Bovine Tuberculosis in Northern Ireland:

Surveillance, Biosecurity and Farmers' Attitudes

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Runder Tuberculosis in Noord-Ierland: Bewaking, Biosecurity en het Standpunt van Veehouders

(met een samenvatting in het Nederlands)

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“Questioning is the door of knowledge”

- Irish Proverb -

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Chapter 1

Introduction

The Northern Ireland cattle industry

The agricultural industry in Northern Ireland is heavily reliant on cattle, with beef and milk being responsible for 50% of the gross agricultural output. In June 2017 there were approximately 1.7 million cattle in Northern Ireland distributed among approximately 24,000 active herds with the predominant herd type being beef-cow herds (only 19% were classified as dairy farms). Although some large herds are present, the majority of farms are relatively small with almost half of active farms having less than 40 cattle (Department of Agriculture, Environment and Rural Affairs, 2017).

Bovine tuberculosis

Bovine tuberculosis (bTB) is a chronic, infectious disease with a global distribution. In Northern Ireland and other parts of the British Isles it is one of the most important endemic diseases facing the cattle industry. It is a zoonotic disease with implications for animal health and significant economic consequences for the Government as well as being the cause of great emotional and financial distress to the farmers whose herds are affected (Abernethy et al., 2006).

Bovine tuberculosis is mainly caused by *Mycobacterium bovis*, an acid-fast, aerobic, slow growing bacterium with its optimal living temperature at 37 °C. *Mycobacterium bovis* is part of the *Mycobacterium tuberculosis* complex, a genetically related group of *Mycobacterium* species that can cause tuberculosis (Rodriguez-Campos et al., 2014). In countries with an eradication programme most infected cattle are identified before development of any clinical symptoms. In the late stages of disease common presenting signs include emaciation, fluctuating fever, weakness, inappetence, coughing, dyspnoea and enlarged lymphnodes (Une and Mori, 2007). The most commonly identified pathological change in infected animals are tuberculous lesions which can mainly be found in lymph nodes associated with the respiratory tract (Neill et al., 2001).

Cattle are the most common host of *M. bovis* but the bacterium can also be isolated from humans, goats, cats, dogs, pigs, buffalo, badgers, possums, deer, bison and many other mammals (Pesciaroli et al., 2014). Some susceptible species are reservoir hosts which can maintain infection and can act as a source of infection. Examples of this type of host are badgers or wild deer on the British Isles (Broughan et al., 2013).

Although there are numerous ways in which bTB spreads between cattle including horizontal spread by inhalation and ingestion and vertical transmission, aerosol infection is commonly accepted as the most important route based on the distribution of tuberculous lesions in infected animals. Furthermore it is known that significantly larger numbers of *M. bovis* are required to cause infection by ingestion compared to aerosol infection (Neill et al., 2001).

Mycobacterium bovis is usually transmitted to humans by consumption of raw infected cow's milk, but it can spread via aerosol droplets as well. In developed countries human infections are very rare nowadays due to milk pasteurisation (Kleeberg, 1984). However in developing countries where pasteurisation is not routine and other diseases such as the human immunodeficiency virus (HIV) are associated with a greatly increased risk of overt disease, it is believed that there is an increased risk of *M. bovis* infections in humans (O'Reilly and Daborn, 1995).

Wildlife

The role of wildlife as a reservoir of infection has been demonstrated globally; Eurasian badgers and wild deer in the British Isles (Delahay et al., 2007; Corner et al., 2011), possums in New Zealand (Nugent, 2011), African buffalos in South Africa (Renwick et al., 2007) and wild boar in Spain (Gortazar et al., 2011). The evidence base for badgers playing a role in bTB epidemiology includes the known susceptibility of badgers for bTB, the plausibility of transmission routes, evidence based on molecular strain typing (Smith et al., 2006; Skuce et al., 2010) and the outcomes of badger culling trials in Great Britain and the Republic of Ireland (as reviewed by Allen et al., 2011). The first research conducted in Northern Ireland indicating that badgers play a role in the transmission of bTB dates back to 1999 (Denny and Wilesmith, 1999). However, to date there are still uncertainties in relation to quantification and routes of transmission (direct/indirect) of bTB between badgers and cattle but reducing the possibility of badgers transmitting bTB to cattle has caused a significant reduction of in the breakdown risks in herd in the Republic of Ireland in areas where badgers were culled (Byrne et al., 2014). As badgers are a legally protected species under the Wildlife (NI) Order of 1985 and under Appendix III of the Berne Convention, any interference of badgers and their setts is not straight forward and needs extensive justification and licensing. This is one of the main reasons (along with cost) why currently there is no routine badger bTB intervention in Northern Ireland; there is a current research project in a 100 km^2 zone in county Down based on a Test Vaccinate or Remove approach[1]. There is passive surveillance for bTB in badgers ongoing since 1998 in the form of a road traffic accident survey covering all areas of Northern Ireland (Abernethy et al., 2003; Abernethy et al., 2011; Courcier et al., 2018). The badger TB prevalence in Northern Ireland based on currently available data is estimated to be 15.3% (95% Confidence Interval 13.1-17.5) (Courcier et al., 2018).

Surveillance/diagnosis

As previously stated in countries where eradication programmes are in place, infected cattle rarely show clinical symptoms as they are removed prior to these developing. Furthermore the symptoms of bovine tuberculosis can take months to develop and infections can remain dormant for years until reactivation due to stress, secondary illness or old age may occur (Cousins, 2001). Surveillance for bTB is therefore mainly based on early diagnosis by detection of immune responses or by routine abattoir surveillance.

A compulsory bTB eradication programme has been in operation in Northern Ireland since 1959 (Abernethy et al., 2006). The main ante-mortem diagnostic test for bTB detection in Northern Ireland is the Single Intradermal Comparative Cervical Tuberculin (SICCT) test, commonly referred to as the ‘skin test’, which is the statutory test performed within European Member States as part of eradication programmes under the 64/432/EEC directive (as amended). In Northern Ireland all

[1] Department of Agriculture, Environment and Rural Affairs: Test, Vaccinate or Remove (TVR) wildlife intervention research. On line available on: https://www.daera-ni.gov.uk/articles/test-and-vaccinate-or-remove-tvr-wildlife-intervention-research

cattle older than 6 weeks are tested routinely on an annual basis using the SICCT test with both private veterinary practitioners and government veterinary officers undertaking the testing (Abernethy et al., 2006). The SICCT test encompasses two separate injections of avian and bovine purified protein derivative (PPD) tuberculin intradermally into defined sites on the neck of cattle. The test is read 72 hours thereafter by comparing the relative millimetre increase in skin fold thickness relating to an *in vivo* cell mediated response to each tuberculin at each injection site. The interpretation of the test depends on the cut-off values applied with the 'standard interpretation' referring to the bovine reaction being ≥4 mm and exceeding the avian reaction by more than 4 mm, and 'severe interpretation' referring to the bovine reaction being ≥2 mm) and exceeding the avian reaction by more than 2 mm (Monaghan et al., 2004; Clegg et al., 2011). The SICCT test method has a good average specificity (99%-100%) but a relatively poor average sensitivity (51-80%) at standard interpretation (De La Rua Domenech et al., 2006) resulting in the test being generally prone to false negatives. Animals that react positively to the SICCT test are compulsory removed and are subjected to detailed examination at slaughter and where infection has not been confirmed previously in the disease incident, lymph-node and other appropriate tissue samples are collected for histological and/or bacteriological confirmation. Once test-positive cattle are removed the herd is subjected to animal movement restrictions and short interval testing with a broader application of severe interpretation of the SICCT test. Furthermore, contact cattle or herds are identified and repeatedly SICCT tested subject to risk assessment (Abernethy et al., 2006).

In addition to the SICCT test the Northern Ireland bTB programme uses the gamma interferon (IFNγ test) in a limited number of herds. The IFNγ test is a European Commission approved ancillary test to the SICCT test in order to maximize the detection of bTB infected animals (Regulation EC/1226/2002 amending Annex B to Directive 64/432/EEC). The test is carried out in two steps with the first step being a short-term culture of whole blood in the presence and absence of mycobacterial antigens. The second step is a capture enzyme linked immuno-absorbent assay (ELISA) for the measurement of IFNγ in the plasma of the blood cultures (Ryan et al., 2000). The IFNγ test has a better sensitivity (88-94%), but poorer specificity (85-98%) making it more prone to false positives (De La Rua Domenech et al., 2006) compared to the SICCT test. Participation in IFNγ testing is voluntary for selected herds as is the removal of IFNγ positive animals (Lahuerta-Marin et al., 2015).

Post-mortem inspection of all cattle routinely slaughtered is undertaken for meat hygiene purposes and, in relation to bTB, acts as an ancillary surveillance system. Cattle are inspected for visible bTB like lesions in accordance with EC Directive 854/2004. When such lesions are detected in animals that are not-compulsory slaughtered under the bTB programme, a disease control response is automatically initiated including animal movement restrictions from the source herd of the suspect animal. These restrictions are kept in place until confirmation by histology or bacteriological culture or otherwise of M. bovis being the cause of the lesion is completed (Pascual-Linaza et al., 2016).

Bovine tuberculosis statistics in Northern Ireland

A comprehensive lifetime history of between-herd animal movement and SICCT testing is available for each animal in Northern Ireland on the central animal health database of the Department of Agriculture, Environment and Rural Affairs (DAERA) (Houston, 2001).

Looking at the annual bTB herd incidence there was a rising trend in disease levels continuing into the early 2000s rising to 9.92% in 2002 after which it fell to 5.35% in 2007. Over the years 2007-2010 herd incidence remained relatively level and in 2010 the annual incidence was 5.12%, its lowest level since 1998. A sharp rise has taken place in 2011 and is still continuing with the current bTB herd incidence being 9.38% (July 2018). In July 2018, 89.8% of herds in Northern Ireland were officially tuberculosis free (Department of Agriculture, Environment and Rural Affairs, 2018).

Objectives and outline of the dissertation

Failure to eradicate bTB in Northern Ireland even though a compulsory bTB programme has been in place for nearly sixty years has been attributed to a range of factors. These factors include high herd and cattle density, small farm unit size and the extensive use of outlying rented pasture which all facilitate cattle to cattle spread exacerbated by significant between herd movement and winter housing of cattle. Further to these farm management related factors there is added difficulty of particularly the badger population being a wildlife reservoir for bTB (Abernethy et al., 2006). In addition human factors such as the role of farmers' attitudes towards bTB and its control are considered to have an important bearing on the success of the bTB scheme (Robinson, 2015).

This dissertation aims to address some of the difficulties that the Northern Ireland bTB programme has faced particularly focussing on improved used of surveillance (both at abattoir level and SICTT test level), biosecurity and farmers' attitudes. The thesis findings are written up in five chapters based on four separately conducted studies.

Chapter 2 focuses on cattle that are positive to the SICCT test and are therefore subsequently slaughtered. The objective of this study was to get a better insight into the risk factors that affect the development of visible lesions at post-mortem examination or positive laboratory tests in these particular animals. This is important as failure of confirming disease can lead to a less stringent follow-up regime. Practically it is useful to determine which risk factors are important in the identification of infected animals. Once we are aware which risk factors are present for the purpose of abattoir surveillance, more vigilance during the post mortem examination for animals in these categories can be proposed.

Chapter 3 and 4 are based on a field study conducted in the South-East of Northern Ireland aiming to get a better understanding of farm management and badger related biosecurity measures that could potentially have a protective effect on bTB breakdowns. In addition this study aimed to evaluate the level of adoption of biosecurity measures by farmers and their attitudes towards bTB and its control.

Chapter 5 evaluates the impact of the number of SICCT test positive animals and confirmation of infection on the risk of future bTB breakdown. This is important as number of reactors and confirmation both have a direct impact on the follow-up regime after disclosure of positive animals.

Chapter 6 applies Bayesian latent class analyses to estimate the test characteristics of the SICCT test and post-mortem surveillance taking into account explanatory factors. Knowing the sensitivity and specificity of these diagnostics within a Northern Ireland setting is not only of importance in its own right but will feed in to future research projects.

Finally chapter 7 provides a general discussion in relation to bovine tuberculosis in Northern Ireland (and beyond). Furthermore it will focus on the impact of the research conducted for this thesis.

References

Abernethy, D.A., Denny, G.O., Pfeiffer, D.U., Wrigley, K. (2003). Survey for *Mycobacterium bovis* infection in road-traffic-accident badgers in Northern Ireland. Proceedings of the 10th International Symposium for Veterinary Epidemiology and Economics, Vina de Mer, Chile, 18–21 November 2003

Abernethy, D.A., Denny, G.O., Menzies, F.D., McGuckian, P., Honhold, N., Roberts, A.R. (2006): The Northern Ireland programme for the control and eradication of *Mycobacterium bovis*. Veterinary Microbiology 112, Issue 2-4, 231-237

Abernethy, D.A., Walton, E., Menzies, F.D., Courcier, E.A., Robinson, P. (2011): *Mycobacterium bovis* surveillance in European badgers (*Meles meles*) killed by vehicles in Northern Ireland: an epidemiological evaluation- presented at the 2011 International Conference on Animal Health Surveillance (ICAHS), Anses, France, 17-20 May 2011

Allen, A.R., Skuce, R.A., McDowell, S.W.J. (2011): Bovine TB a review of badger-to-cattle transmission. Report for the Department of Agriculture, Environment and Rural Affairs. On line available at: https://www.daera-ni.gov.uk/sites/default/files/afbi-literature-review-tb-review-badger-to-cattle-transmission.pdf Accessed on 05/06/2018

Broughan, J. M., Downs, S.H., Crawshaw, T.R., Upton, P.A., Brewer, J., Clifton-Hadley, R.S. (2013): Mycobacterium bovis infections in domesticated non-bovine mammalian species. Part 1: Review of epidemiology and laboratory submissions in Great Britain 2004-2010. Veterinary Journal, 198(2), 339-345

Byrne, A.W., White, P.W., McGrath, G., O’Keeffe, J., Martin, S.W., 2014. Risk of tuberculosis cattle herd breakdowns in Ireland, effects of badger culling effort, density and historic large-scale interventions. Veterinary Research 45(1), 109

Clegg, T.A., Duignan, A., Whelan, C., Gormley, E., Good, M., Clarke, J., Toft, N., More, S.J. (2011): Using latent class analysis to estimate the test characteristics of the γ-interferon test, the single intradermal comparative tuberculin test and a multiplex immunoassay under Irish conditions. Veterinary Microbiology 151, 68-76

Corner, L. A., Murphy, D., Gormley, E. (2011): *Mycobacterium bovis* infection in the Eurasian badger (*Meles meles*): the disease, pathogenesis, epidemiology and control. Journal of Comparative Pathology 144, 1-24

Courcier, E.A., Menzies, F.D., Strain, S.A.J., Skuce, R.A., Robinson, P.A., Patterson, T., McBride, K.R., McCormick, C.M., Walton, E., McDowell, S.W.J., Abernethy D.A. (2018): *Mycobacterium bovis* surveillance in Eurasian badgers (*Meles meles*) killed by vehicles in Northern Ireland between 1998 and 2011. Veterinary Record 182(9), 259. doi: 10.1136/vr.103934

Cousins, D.V. (2001): Infection and control in domestic livestock. *Mycobacterium bovis.* Revue Scientifique et Technique (International Office of Epizootics) 20, no. 1, pp. 71-85. PMid:11288521

Delahay, R.J., Smith, G.C., Barlow, A.M., Walker, N., Harris, A., Clifton-Hadley, R.S., Cheeseman, C.L. (2007): Bovine tuberculosis infection in wild mammals in the South-West region of England: A survey of prevalence and a semi-quantitative assessment of the relative risks to cattle. Veterinary Journal 173, 287-301

De la Rua-Domenech, R., Goodchild, A.T., Vordermeier, H.M., Hewinson, R.G., Christiansen, K.H., Clifton-Hadley, R.S. (2006): Ante mortem diagnosis of tuberculosis in cattle: a review of the tuberculin tests, gamma-interferon assay and other ancillary diagnostic techniques. Research in Veterinary Science 81:190–210. doi: 10.1016/j.rvsc.2005.11.005

Department of Agriculture, Environment and Rural Affairs (2017): Statistical review of Northern Ireland Agriculture 2017. On line available on: https://www.daera-ni.gov.uk/sites/default/files/publications/daera/Agricultural%20Census%20in%20Northern%20Ireland%202017.pdf Accessed on 05/06/2018.

Department of Agriculture, Environment and Rural Affairs (2018): Bovine tuberculosis statistics for Northern Ireland. On line available on: https://www.daera-ni.gov.uk/publications/tuberculosis-disease-statistics-northern-ireland-2018 Accessed on 05/06/2018.

Denny, G.O., Wilesmith, J.W. (1999): Bovine tuberculosis in Northern Ireland: a case-control study of herd risk factors. Veterinary Record 144, 305-310

Gortazar, C., Vicente, J., Boadella, M., Ballesteros, C., Galindo, R.C., Garrido, J. (2011): Progress in the control of bovine tuberculosis in Spanish wildlife. Veterinary Microbiology 151, 170-178

Houston, R. (2001): A computerized database system for bovine traceability. Revue Scientifique et Technique 20 (2), 625-661

Kleeber, H.H. (1984): Human tuberculosis of human origin in relation to public health. Revue Scientific et Technique of the Office International des Epizooties (Paris) 3 (1), 11-32

Lahuerta-Marin, A., Gallagher, M., McBride, S., Skuce, R., Menzies, F.D., McNair, J., McDowell, S.W.J., Byrne, A.W. (2015): Should they stay or should they go? Relative future risk of bovine tuberculosis for interferon-gamma test-positive cattle left on farms. Veterinary Research 46:90, doi 10.1186/s13567-015-0242-8

Monaghan, M.L., Doherty, M.L., Collins, J.D., Kazda, J.F., Quinn, P.J. (1994). The tuberculin test. Veterinary Microbiology 40, 111-124

Neill, S.D., Bryson, D.G., Pollock, J.M. (2001): Pathogenesis of tuberculosis in cattle. Tuberculosis 81, 79-88

Nugent, G., Yockney, I.J., Whitford, E.J. (2011): Intraspecific transmission of *Mycobacterium bovis* among penned feral pigs in New Zealand. Journal of Wildlife Diseases 47, 364-372

O'Reilly, L.M. and Daborn, C.J. (1995): The epidemiology of *Mycobacterium bovis* infections in animals and humans, Tubercle and Lung Disease 76, Suppl. 1, 1-46

Pascual-Linaza, A.V., Gordon, A.W., Stringer, L.A., Menzies, F.D. (2016): Efficiency of slaughterhouse surveillance for the detection of bovine tuberculosis in cattle in Northern Ireland. Epidemiology and Infection 145(5), 995-1005

Pesciaroli, M., Alvarez, J., Boniotti, M.B., Di Marco, V., Marianelli, C., Pacciarini, M., Pasquali, P. (2014): Tuberculosis in domestic species. Research in Veterinary Science 97, S78-S85

Renwick, A.R., White, P.C., Bengis, R.G. (2007): Bovine tuberculosis in southern African wildlife: a multi-species host-pathogen system. Epidemiology and Infection 135, 529-540

Ryan, T.R., Buddle, B.M., de Lisle, G.W. (2000): An evaluation of the gamma interferon test for detecting bovine tuberculosis in cattle 8 to 28 days after tuberculin skin testing. Research in Veterinary Science 69, 57-61

Robinson, P.A. (2015): A history of bovine tuberculosis eradication policy in Northern Ireland. Epidemiology and Infection 143(15), 3182-3195

Rodriguez-Campos, S. Smith, N.H., Boniotti, M.B., Aranaz, A. (2014): Overview and phylogeny of *Mycobacterium tuberculosis* complex organisms: Implications for diagnostic and legislation of bovine tuberculosis. Research in Veterinary Science 97, S5-S19

Smith, N.H., Gordon, S.V., de la Rua-Domenech, R., Clifton-Hadley, R.S., Hewinson, R.G. (2006): Bottlenecks and Broomsticks: The molecular evolution of *Mycobacterium bovis.* Nature Reviews Microbiology 4, 670-681

Skuce, R.A., Mallon, T.R., McCormick, C.M., McBride, S.H., Clarke, G., Thomson, A., Cousens, C., Gordon, A.W., McDowell, S.W.J. (2010): *Mycobacterium bovis* genotypes in Northern Ireland: herd-level surveillance (2003 to 2008). Veterinary Record 167, 684–689

Une, Y., Mori, T. (2007): Tuberculosis as a zoonosis from a veterinary perspective. Comparative Immunology, Microbiology and Infectious Diseases 30, 5-6, 415-425

Chapter 2

Risk factors for visible lesions or positive laboratory tests in bovine tuberculosis reactor cattle in Northern Ireland

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Abstract

An observational case-control study was conducted to investigate risk factors for confirmed bovine tuberculosis (bTB) infection in cattle reacting positively to the single intradermal comparative cervical test (SICCT) in Northern Ireland in the years 1998, 2002 and 2006.

Macroscopic lesions were detected at slaughter (positive visible lesion (VL) status) in 43.0 % of reactor cattle, whilst 45.3 % of those sampled were confirmed as bTB positive due to the presence of lesions or positive histopathology/mycobacterial culture (positive bTB status). In 97.5 % of the reactors, the VL status and bTB status were either both negative or both positive. Generalized linear mixed model analyses were conducted on data of 24,923 reactor cattle with the variables herd identifier, local veterinary office (DVO) and abattoir being used as random effects within all the models generated at univariable and multivariable level. The other variables within the dataset were used as fixed effects. Significant risk factors associated with VL status and bTB status at multivariable level ($p<0.05$) included age at death, breed, sex, test year, net increase in skin thickness at bovine tuberculin injection site, epidemiological status of skin test, total number of reactors at the disclosure test, mean herd size and prior response to the skin test.

These risk factors are likely related to the time since infection, the strength of the challenge of infection and the susceptibility of the animal. These findings are important as the detection of visible lesions and the confirmation of bTB are an integral part of the overall bTB control programme in Northern Ireland and the veterinary meat inspection and hygiene programme. The visible lesion status and bTB status of an animal can affect the way in which bTB breakdowns are managed, since failure to detect visible lesions and recovery of *M. bovis* can lead to a less stringent follow-up after other risk factors have been taken into account.

Introduction

Bovine tuberculosis (bTB) is a chronic, infectious and zoonotic disease of domestic and wild animals caused by *Mycobacterium bovis*. Transmission of infection to cattle occurs from the environment (e.g. faeces), wildlife (e.g. badger), humans and cattle (Good and Duignan, 2011). The airborne route is the most important route of transmission in cattle (Morris *et al*., 1994) with 90-95 % of the primary foci being located in the respiratory tract (Palmer *et al.,* 1999; Quinn *et al.,* 2004). Following aerosol exposure and phagocytosis, infected macrophages enter the lymphatic system and are carried to the lymph nodes. This engulfment with macrophages in turn will activate other macrophages and draw helper T-cells to the area. Activated T-cells then proceed to kill macrophages infected with mycobacteria leading to destruction of the surrounding tissue, which in combination with the dead or dying macrophages creates caseous necrosis forming a granuloma or lesion. Not only does this lesion or granuloma create a micro-environment in which infection can be controlled, it also provides the mycobacterium with a niche in which it can survive (Miranda *et al.*, 2012). The evolution of lesions is dynamic and different between individuals (Grosset, 2003).

Clinical signs of bTB (i.e. coughing and weight loss) are now rarely seen in the United Kingdom due to the slow progression of disease and the Government's compulsory testing and slaughter programme. However, despite this compulsory scheme to control bTB being in place since 1959, bTB is still endemic and of high financial importance in Northern Ireland (Anon., 2011). The cattle density is high, most cattle trade takes place at livestock sales, winter housing is common and sixty percent of farms in Northern Ireland have multiple premises. All these factors promote movement and cattle-to-cattle contact (Abernethy *et al*., 2006). In addition, Northern Ireland has a wildlife reservoir for bTB in Eurasian badgers (*Meles meles*) (Denny and Wilesmith, 1999; Abernethy *et al.*, 2011).

The single intradermal comparative cervical test (SICCT) is the primary ante-mortem diagnostic tool for bTB in cattle. Estimates of the sensitivity of the SICCT range from 68-95% depending on the potency and dose of tuberculin administered, the post-infection interval, desensitisation, deliberate interference, post-partum immunosuppression and observer variation (Monaghan *et al*., 2004; De la Rua-Domenech *et al*., 2006).

In Northern Ireland, all cattle over 6 weeks of age are tested annually with the SICCT by government veterinarians or private veterinary practitioners. In addition there is computerised tracing of contact herds and cattle, short interval testing of herds contiguous to outbreaks and compulsory slaughter of positive cattle. On disclosure of reactors to the SICCT or tuberculous lesions at routine post mortem inspection, herds are restricted from moving animals, except direct to slaughter, until they have passed several tests at intervals of 42 to 60 days (Abernethy *et al*., 2006).

In developed countries, the main purpose of meat inspection in relation to bTB is to act as an ancillary surveillance system and it is an essential component of the overall control programme (Olea-Popelka *et al*., 2008). The sensitivity of gross post-mortem examination depends on the method employed and the anatomical sites examined. The detection rate of visible lesions varies significantly between abattoirs (Frankena *et al.,* 2007; Olea-Popelka *et al.*, 2012; Shittu *et al.*, 2013; Wright *et al.,* 2013). In Northern Ireland all reactors with visible lesions are subjected to histology examination of which the majority shows tuberculoid granulomata. These samples are subsequently reported as having a positive bTB status. Those samples that do not demonstrate tuberculoid granulomata on histological examination are subjected to bacterial culture. Lymph node tissue samples from reactors without visible lesions are trimmed, serial sliced and examined for lesions. If no lesions are found these samples are subjected to bacteriological culture only. The likelihood of culturing *M. bovis* is greatly increased by sampling from macroscopic lesions ("Visible lesions" or "VL") and/or by thinly slicing lungs of infected cattle (DEFRA, 2007; OIE, 2009). Lack of macroscopic lesions ("Non visible lesions" or NVL) could be due to early infection, the poor sensitivity of the post-mortem examination or infection with Mycobacteria other than *M. bovis* (Corner, 1994).

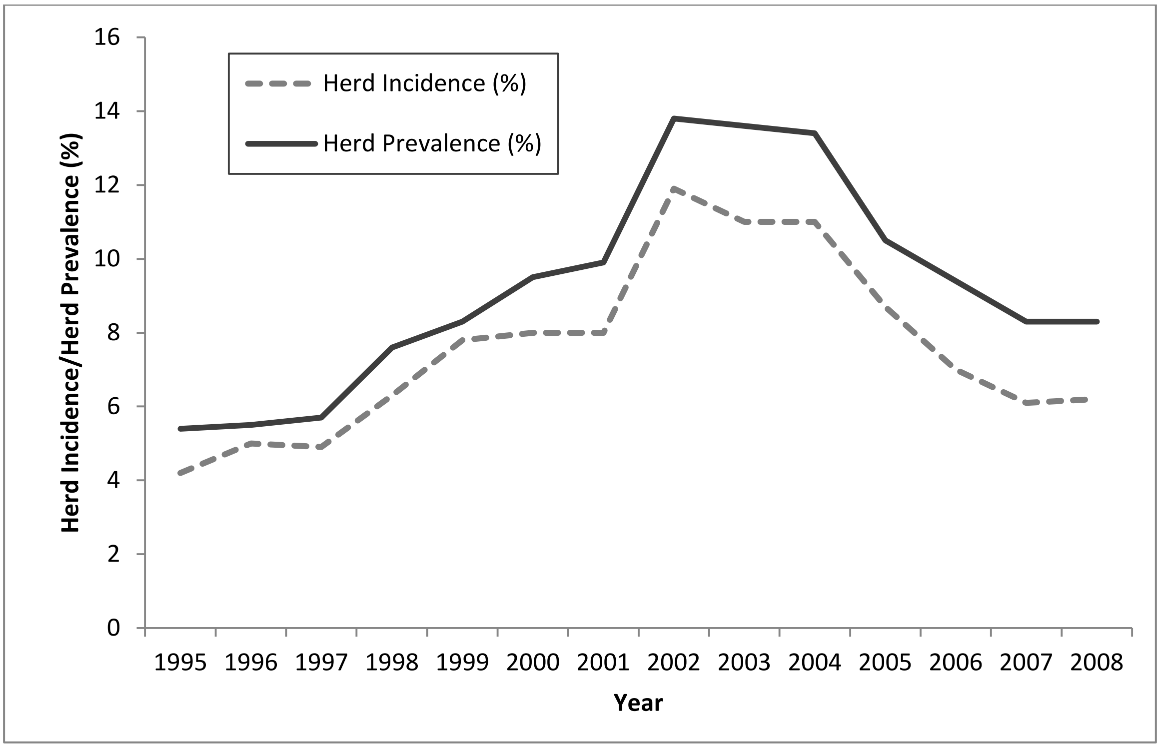
The purpose of this study was to determine the risk factors associated with the presence of visible bTB lesions or positive laboratory tests (i.e. histology and culture) in cattle that reacted positively to the SICCT with the aim of being able to use this information to inform and improve disease control.

Materials and methods

Study population

The units of interest for this study were cattle that were removed from the herd due to a positive reaction to the SICCT. Reactor cattle were selected from three years, reflecting differing bTB trends; 1998 (increasing incidence), 2002 (peak incidence) and 2006 (reducing incidence) (Figure 1). The total cattle population in Northern Ireland was approximately 1.7 million in each of the three study years (DARD, 2011) and the total number of reactor animals in those three years was 31,883.

Figure 1: Bovine tuberculosis herd incidence and herd prevalence in Northern Ireland between 1995 and 2008



Data source

Data were extracted from the central animal health database (APHIS) of the Department of Agriculture and Rural Development for Northern Ireland (DARD), which contains the details of all individual cattle, cattle holdings, cattle movements and cattle tuberculosis and brucellosis tests in Northern Ireland since 1988 (Houston, 2001). Complete information was available on the animal health data base for reactor animals in relation to their place of slaughter for the years 2002 and 2006. In 2002, 99.9% of reactor animals were slaughtered in one of three abattoirs. These reactors were nearly evenly distributed over these three abattoirs (respectively 27.7%, 33.0% and 39.2%). However, in 2006 one of these three abattoirs became the destination for 91.7% of reactor animals. Post mortem inspection for evidence of bTB lesions in reactor animals includes the examination of the lymphnodes of the head, chest and mesenterium, lungs, pleura, peritoneum, prescapular lymphnodes, popliteal lymphnodes, iliac lymphnodes and the precrural lymph nodes. Not all reactor animals are subjected to laboratory testing depending on the number of reactors in the herd and lesion status of the animals. For every breakdown samples for up to 5 reactors without VL and 3 VL reactors are subjected to laboratory testing. The laboratory samples of lesioned reactor animals usually consist of at least one lesion, whereas for non-lesioned reactor animals the samples are usually the retro-pharyngeal, bronchial and mediastinal lymph nodes which are bulked together by herd. When lesions are negative on histological examination or when no lesions are found, bacteriological culture is performed.

Study design

The study design was a classical, observational case-control study. Two analyses were performed. In the first analysis, cases were reactors to the SICCT with VL detected at slaughter, while controls were reactors with no visible lesions (NVL) detected in the abattoir. In the second analysis, cases were reactors with positive bTB status either due to having lesions or positive laboratory tests (bacteriological culture or histopathology), and the controls were reactors with negative laboratory test results. The outcome modelled was the VL status of the reactor animal (first analysis) or its bTB status (second analysis) after histopathology and/or culture. Potential risk factors were explored at three levels: host, test and herd.

The net bovine rise was calculated as the increase (in millimetres) at the bovine tuberculin injection site greater than any increase at the avian tuberculin injection site when measured after 72 hours (as per Council Directive 64/432/EEC, Annex B). The risk status of the disclosure test was divided into 3 types: routine (in situations where no risk of bTB infection is suspected to be in the herd), at risk (in situations where the herd is at increased risk of having bTB infection) and restricted (in situation where reactors or animals with lesions at routine slaughter have been found or the herd is at high risk of having bTB infection). The bTB history in the previous herd was defined as bTB being recorded in at least one herd through which the reactor animal moved prior to being in the disclosure herd and which occurred whilst the reactor animal resided in the herd. Herd size was the average number of cattle tested at herd level tests in the three years prior to the disclosure test. The previous testing history of the reactor animal was taking into account in the analyses by evaluating whether an animal had had an inconclusive (IC) reaction to the SICCT before. An inconclusive reaction to the SICCT can be defined as an increase in skin fold thickness of more than 2 mm and less than 4mm under standard interpretation of the test without diffuse oedema, exudation, necrosis, pain or inflammation of the lymphatic ducts in that region or of the lymph nodes (as per Council Directive 64/432/EEC, Annex B). The previous bTB history of neighbouring herds was taken into account by evaluating whether the animal had a history of being subjected to a Lateral Check Test (LCT) in the previous year. LCTs are carried out on herds that graze land adjacent to that of a herd with a confirmed bTB breakdown. The number of purchased animals was represented by the number of animals entering the herd in the three years prior to the disclosure herd test.

Data analysis

Microsoft Access 2007 was used for data manipulation while the descriptive analyses were undertaken in ‘R’ version 2.15.2. (The R Foundation for Statistical Computing). Statistical significance was defined as $p<0.05$. The variables were tabulated and analysis was carried out for each of the variables included in the study. Odds ratios with 95% confidence intervals (95% CI) were calculated to examine the association between the risk factors and the VL status and bTB status of the reactor animals. The statistical analyses were conducted in GenStat (14.1.0.5943) using generalized linear mixed modeling with the variables herd identifier, local veterinary office (DVO) and abattoir being used as random effects within all the models generated at univariable and multivariable level. The other variables within the dataset were used as fixed effects. Possible first order interactions between the independent variables were explored. The models developed for both VL status and bTB status as being the outcome were firstly checked for significance at univariable level. A *p*-value <0.15 was considered significant. Thereafter a multivariable model of the fixed effects was generated by sequentially adding all the significant variables. This full model was then refined by dropping the non-significant terms with $p>0.05$ for the final multivariable model.

Results

Missing data

A total of 31,883 reactor animals were present in the database. Complete data on all variables were available for 24,923 reactors (78.2%). Most of the missing data were based on incomplete details on the abattoir the reactor animal was slaughtered in (n=4,895; all in 1998) and missing VL status and/or bTB status (n=1,632) of which the majority (n=1,089; 66.7%) were based on reactors in 1998. Univariable and multivariable analyses were conducted on the reactors (n=24,923) for which all the data were available to be able to take account of abattoir as a random effect. Analyses were repeated on 29,818 reactors for which all data were available except the details of the abattoirs that the reactor animal was slaughtered in. This analysis was conducted in the same manner as the analysis described previously except that only herd identifier and local veterinary office (DVO) were added as a random variable. This analysis gave similar outcomes to the results described in the current article.

Dependent variables

Table 1 shows the breakdown of reactors into 4 groups based on their VL and bTB status (n=30,251). Cohen's Kappa statistics of 0.949 (SE=0.002) showed almost perfect agreement between VL status and bTB confirmation in these animals (Landis and Koch, 1997).

Table 1: Distribution of the visible lesion status and bTB confirmation status of 30,251 reactor animals in 1998, 2002 and 2006 in Northern Ireland

	VL status*	**NVL status****	**Total**
bTB status^ positive	42.87% *(n=12,969)*	2.43% *(n=736)*	**45.30%** ***(n=13,705)***
No bTB status^^ negative	0.08% *(n=25)*	54.61% *(n=16,521)*	**54.70%** ***(n=16,546)***
Total	**42.95%** ***(n=12,994)***	**57.05%** ***(n=17,257***	**100.0%** ***(n=30,251)***

* VL status: reactor animal had visible lesions at post mortem

** NVL Status: reactor animal had no visible lesion at post mortem

^ bTB status positive: reactor animal had confirmed disease based on visible lesions at slaughter or positive laboratory tests (histology and/or bacteriological culture)

^^ bTB status negative: reactor animal had no confirmed disease

Descriptive and univariable analysis

The results for the descriptive and univariable analysis are displayed in Table 2.

Table 2: Results of univariable analyses in relation to potential risk factors for visible lesion status and bTB status of reactor animals in Northern Ireland. Herd identifier, local veterinary office (DVO) and abattoir were used as random effect

Variable	**Reactors % n=24,923**	**Visible Lesion Status**		**bTB status**	
		Odds ratio (95%CI)	**P value**	**Odds ratio (95%CI)**	**P value**
<u>**Risk factors at host level**</u>					
Age at death					
Per 10 days increase		0.95 (0.94-0.96)	<0.001	0.95 (0.94-0.96)	<0.001
Breed					
Dairy	45.1%	Baseline	<0.001	Baseline	<0.001
Non-dairy	54.9%	2.29 (2.10-2.49)		2.25 (2.07-2.45)	
Sex					
Bull	3.3%	Baseline	<0.001	Baseline	<0.001
Female	82.1%	1.13 (0.95-1.35)		1.16 (0.97-1.37)	
Bullock	14.6%	1.48 (1.23-1.79)		1.48 (1.23-1.78)	
Test year					
1998	4.5%	Baseline	<0.001	Baseline	<0.001
2002	58.7%	0.53 (0.44-0.64)		0.44 (0.36-0.54)	
2006	36.8%	0.28 (0.23-0.34)		0.25 (0.21-0.30)	
Net Bovine Rise*					
Per 1 mm increase		1.15 (1.14-1.15)	<0.001	1.15 (1.14-1.15)	<0.001
Number of lifetime moves					
0	61.8%	Baseline	0.467	Baseline	0.803
1	21.4%	1.02 (0.97-1.10)		1.02 (0.98-1.10)	
>1	16.7%	0.96 (0.88-1.05)		0.98 (0.90-1.07)	
bTB history in previous herd					
No bTB	18.2%	Baseline	0.085	Baseline	0.200
bTB	20.0%	0.90 (0.81-0.99)		0.92 (0.83-1.01)	
No movement ^	61.8%	N/A^		N/A^	
Previous IC ~					
Not previous IC	96.1%	Baseline	0.130	Baseline	0.177
Previous IC	3.9%	1.13 (0.97-1.31)		1.11 (0.95-1.29)	
LCT # previous year					
Not LCT	45.2%	Baseline	0.061	Baseline	0.080
LCT	54.8%	0.93 (0.87-1.00)		0.94 (0.87-1.01)	

Table 2: continued

Variable	Reactors % n=24,923	Visible Lesion Status		bTB status	
		Odds ratio (95%CI)	**P value**	**Odds ratio (95%CI)**	**P value**
Risk factors at test level					
Risk status disclosure test					
Routine	14.7%	Baseline	<0.001	Baseline	<0.001
At risk	29.4%	0.85 (0.76-0.96)		0.87 (0.77-0.97)	
Restricted	55.9%	0.52 (0.59-0.65)		0.52 (0.47-0.58)	
Total nr of reactors					
Per 5 reactors increase		1.04 (1.02-1.06)	<0.001	1.02 (1.00-1.03)	0.059
Risk factors at herd level					
Mean herd size					
Per 10 animals increase		0.98 (0.98-0.99)	<0.001	0.98 (0.98-0.99)	<0.001
Nr purchased in last 3 years					
Per 10 animals increase		1.00 (0.99-1.00)	0.388	1.00 (0.99-1.00)	0.183

* Increase in mm at the bovine tuberculin injection site greater than any increase at the avian tuberculin injection site when measured after 72 hours
^ 61.9% (n=18,481) of reactor animals remained in their herd of birth until slaughtered and were thus excluded in this part of the analysis.
~ Inconclusive
\# Lateral Check Test

Risk factors at host level

There was a significant association between age at death of the reactor and both the VL status and bTB status. In general, older reactor animals were less likely to have visible lesions or confirmed bTB than younger reactors (odds ratio [OR]=0.95 for both VL and bTB status per 10 day increase in age). Non-dairy breed reactors were more likely to have bTB disclosed than dairy animals (OR=2.29 for VL; OR=2.25 for confirmation), as did female cattle or bullocks when compared to bulls (OR for VL=1.13 (95% CI 0.95-1.35) and 1.48 (95% CI 1.23-1.79) respectively; OR for confirmed bTB=1.16 (95% CI 0.97-1.37) and 1.48 (95% CI 1.23-1.78) respectively). Reactors in 2002 and 2006 were less likely to have VL or confirmed bTB than in 1998 (OR for VL=0.53 and 0.28 respectively; OR for confirmed bTB=0.44 and 0.25 respectively).

Reactor animals were increasingly more likely to have visible lesions or confirmed bTB with increasing net bovine rise (OR=1.15 for both VL and bTB status per 1 mm increase). There was no significant association found between the number of lifetime moves, the bTB history in the previous herd, being inconclusive (IC) or having a history of a LCT in the previous year and the VL status and bTB status of reactor animals.

Risk factors at test level

Reactors at an ‘at risk’ test or a ‘restricted’ test were significantly less likely to have visible lesions or confirmed bTB than those disclosed at a routine test (OR for VL=0.85 and 0.52 respectively; OR for confirmed bTB=0.87 and 0.52 respectively). An increase in the total number of reactors at the disclosure test was associated with an increased odds of having a positive VL status (OR=1.04 for VL status per 5 reactors increase).

Risk factors at herd level

Reactor animals from large herds were less likely to have visible lesions or confirmed bTB (OR=0.98 per 10 animals increase for both VL and bTB status). There was no significant association found between the number of animals purchased in the last 3 years and the VL status and bTB status of reactor animals.

Multivariable analysis

The final multivariable model consisted of the age at death, breed, sex, test year, net bovine rise, previous IC (for VL status only) , risk status disclosure test, total number of reactors at disclosure test (for VL status only) and mean herd size (for bTB status only) (Table 3).

Table 3: Results of multivariable analyses in relation to potential risk factors for visible lesion status and bTB status of reactor animals in Northern Ireland. Herd identifier, local veterinary office (DVO) and abattoir were used as random effects

Variable	**Visible Lesion Status**		**bTB status**	
	Odds ratio (95%CI)	**P value**	**Odds ratio (95%CI)**	**P value**
<u>Risk factors at host level</u>				
Age at death (months)				
Per 10 days increase	0.98 (0.97-0.99)	<0.001	0.98 (0.97-0.99)	<0.001
Breed				
Dairy	Baseline	<0.001	Baseline	
Non-dairy	2.03 (1.86-2.22)		1.94 (1.77-2.12)	<0.001
Sex				
Bull	Baseline	0.004		0.002
Female	1.48 (1.23-1.78)		1.52 (1.26-1.82)	
Bullock	1.27 (1.04-1.55)		1.30 (1.06-1.58)	
Test year				
1998	Baseline	<0.001	Baseline	<0.001
2002	0.45 (0.37-0.55)		0.38 (0.31-0.47)	
2006	0.29 (0.24-0.35)		0.27 (0.22-0.33)	
Net Bovine Rise*				
Per 1 mm increase	1.15 (1.14-1.15)	<0.001	1.15 (1.14-1.15)	<0.001
Previous IC ~				
Not previous IC	Baseline			
Previous IC	1.66 (1.42-1.95)	<0.001		
<u>Risk factors at test level</u>				
Risk status disclosure test				
Routine	Baseline	<0.001	Baseline	<0.001
At risk	1.00 (0.89-1.13)		1.03 (0.91-1.16)	
Restricted	0.69 (0.62-0.78)		0.64 (0.57-0.71)	
Total nr of reactors				
Per 5 reactors increase	1.02 (1.01-1.04)	0.009		
<u>Risk factors at herd level</u>				
Mean herd size				
Per 10 animals increase			1.00 (0.99-1.00)	0.017

* Increase in mm at the bovine tuberculin injection site greater than any increase at the avian tuberculin injection site when measured after 72 hours

~ Inconclusive

Discussion

The aim of this study was to determine the risk factors associated with the presence of visible bTB lesions or positive laboratory tests (i.e. histopathology and culture) in cattle that reacted positively to the SICCT. The visible lesion status and bTB status of an animal can affect the way in which bTB breakdowns are managed, since failure to detect visible lesions and recovery of *M. bovis* can lead to a less stringent follow-up.

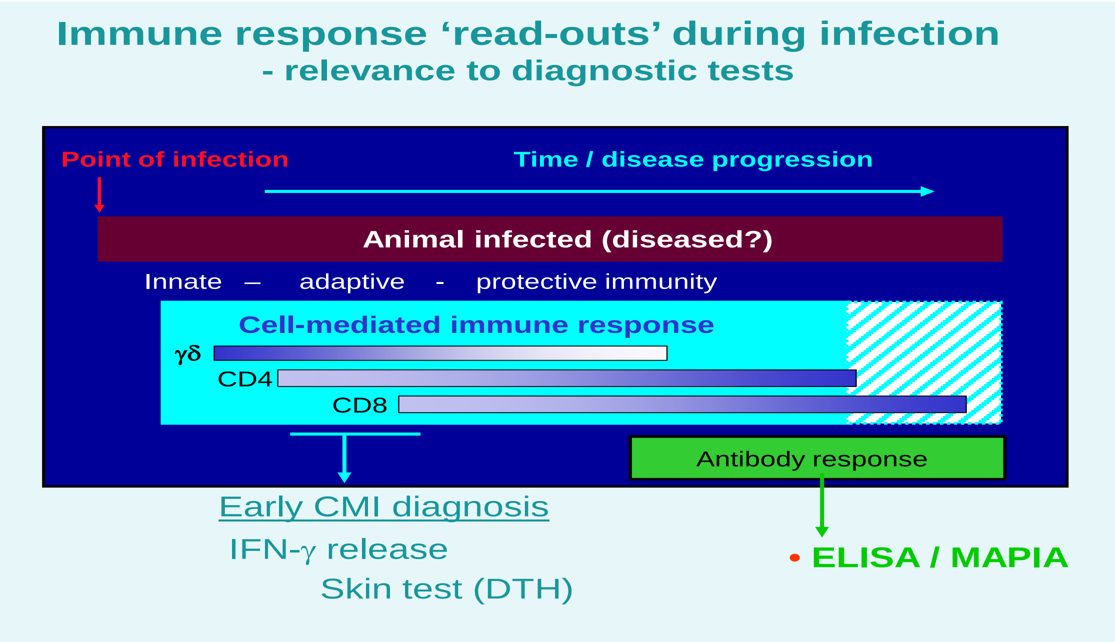
A total of 43.0% percent of the reactor animals in this study had macroscopic lesions consistent with bTB detected at post mortem, while 45.3% had bTB confirmed through the detection of visible lesions or positive laboratory tests. As the specificity of the SICCT is reported to be 99.9% (DEFRA, 2009) this relative large percentage of reactor animals with discordant results between being reactors to the SICCT and having positive VL and/or bTB status is likely to reflect imperfect sensitivity of gross examination of the carcass for lesions and imperfect sensitivity of the laboratory tests (Corner, 1994; Whipple *et al*., 1996).

Several studies (Frankena *et al.,* 2007; Olea-Popelka *et al.*, 2012; Shittu *et al.*, 2013; Wright *et al.,* 2013) described substantial variation in the effectiveness of lesion disclosure and subsequent confirmation of bTB among different abattoirs in the Republic of Ireland, Great Britain and Northern Ireland. This variability was accounted for in the analyses by adding the abattoir where the reactor animals were slaughtered as a random effect at both univariable and multivariable level. In this context it has to be noted that the SICCT is designed to detect the cell mediated immune response (CMI) to bTB infection rather than the signs of disease. This immune response usually develops before visible signs of disease are evident at post-mortem (Figure 2; McNair *et al.* (2006)). There isn't a specific and defined time frame for the development of the different curves in Figure 2 since a number of variables including the route of infection, the infectious dose and host related factors will determine the onset and development of the anti-mycobacterial response (Pollock *et al.*, 2006).

In 97.5 % of the reactor animals, the VL status and bTB status were either both negative or both positive. This nearly perfect agreement between VL and bTB status of the reactor animal can be explained by the knowledge that the likelihood of culturing *M. bovis* from an infected animal is greatly increased by sampling from visible lesions (Neill *et al*., 1992).

Older reactors were less likely to have visible lesions at post-mortem and confirmed bTB than younger animals. Older animals have had a longer time of possible exposure to *M. bovis* and are therefore more likely to be infected (Griffin *et al*., 1996; DEFRA, 2009), however they might also have a longer time to develop lesion after infection, which would contradict this finding. The difference in visible lesion rate would suggest that there is possibly a difference in the immunological status of younger and older cattle. The immune response of cattle following infection with *M. bovis* is displayed in Figure 2. Early in the bovine life there is an immune responsiveness that is developing and changing and has had less exposure to infection in general compared to older animals. For example, there are significantly higher numbers of $CD4^+$ T cells present in adult cattle compared to calves (Wilson *et al.*, 1996; Tanaka *et al*., 2006). Furthermore, although bovine neonates cannot produce B cells in sufficient numbers and at birth numbers are very low, by 6 months of age generation of B cells has developed to the adult level (Tanaka *et al*., 2008). Significantly, the bovine immune system, up to 6 months of age, is influenced by the passive uptake of immune regulators acquired through colostrum (Aldridge *et al*., 1998; Barrington and Parish, 2001). These changes in the immune system could create circumstances that may not permit resolution of infection with *M. bovis* in favour of the host.

Figure 2: Immune responses in cattle following *Mycobacterium bovis* infection by time/disease progression (McNair *et al.*, 2006)



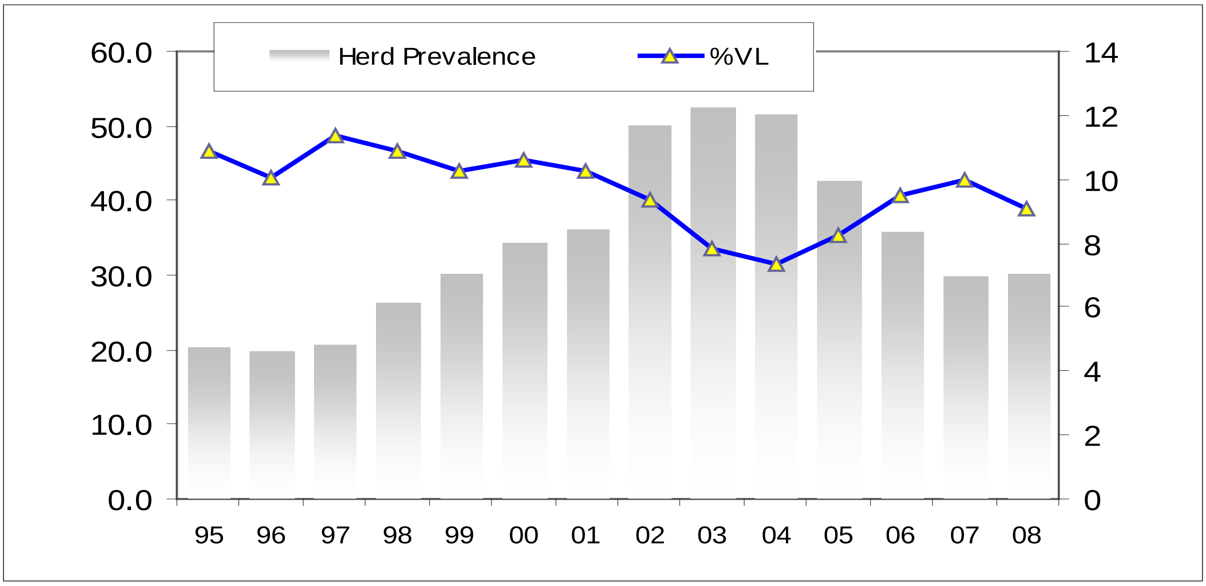
In our study, non-dairy reactor cattle were significantly more likely to have visible lesions and confirmed bTB than dairy reactor cattle for which the reasons are not clear. Similar results were found in studies in England (S.H. Downs, pers. comm.). The difference in skin thickness between dairy and non-dairy breed has been suggested in previous studies as a possible explanation for this (Murray *et al.*, 2012). However, a similar finding was found in cattle that were classified as reactors after being subjected to the gamma interferon test (DEFRA, 2005). Other explanations include genetic/breed differences in susceptibility to bTB (Bermingham *et al*., 2009; Driscoll *et al*., 2011) and the higher within-herd transmission coefficient in dairy herds compared to non-dairy herds (Alvarez *et al.,* 2012) possibly facilitating earlier disclosure of reactors in a herd giving reactor animals less time to develop visible lesions.

Bulls were significantly less likely to have visible lesions and confirmed bTB for which the reason is not clear. Possibly it is related to the under representation of bulls in the current study or the fact that it is easier to miss lesions in a bigger carcass.

In 2002 and 2006, reactor animals were less likely to have visible lesions or confirmed bTB than in 1998. In 1998, the bTB incidence was rising in Northern

Ireland, with a peak incidence in 2002 and decreasing incidence in 2006. In a previous study covering the same time period the visible lesion rate was shown to be converse to the bTB herd prevalence (Figure 3; D.A. Abernethy, pers. comm.).

Figure 3: Percentage of reactors with visible lesions (%VL) and herd prevalence between 1995 and 2008 in Northern Ireland (D.A. Abernethy, pers. comm.)



The left Y-axis shows the VL%; the histogram and right Y-axis show herd prevalence.

Possibly increased short interval testing due to the increase of bTB breakdowns after 1998 and an increased proportion of animals being removed on severe interpretation has lead to animals having less time to develop visible lesions before being disclosed as reactors.

The multivariable model shows that an increase in the net bovine rise significantly increased the odds of reactors having visible lesions or confirmed bTB. This finding is consistent with other studies for example Cassidy *et al*. (1998) and Bermingham *et al.* (2009) who stated the contemporaneous development of lesions and cellular immune response.

There was no significant association between the number of lifetime moves or the number of animals purchased in the last three years and the VL status and bTB status of reactor animals in this study. The importance of movement and purchase of cattle as a risk factor to bTB infection has been supported by several studies (Gilbert *et al*., 2005; Ramirez-Villaescusa *et al.,* 2009; Ramirez-Villaescusa *et al*., 2010).

Abernethy *et al.* (2000) found that there was no significant association between movements through livestock markets and risk of bTB. However these studies have looked at the risk of bTB breakdown in relation to animal movement and not at the risk of subsequently developing visible lesions.

Bovine TB history in previous herds (Olea-Popelka *et al*., 2004; Green and Cornell, 2005; Carrique-Mas *et al.,* 2008) as well as the presence of contagious neighbours with confirmed bTB breakdown, as is the case for cattle that are present at a LCT (Denny and Wilesmith, 1999; Olea-Popelka *et al*., 2004; Ramirez-Villaescusa *et al.,* 2010), are well described bTB risk factors for bTB breakdown. There was however no significant association found between animals that were present at a LCT or which came from a herd with a bTB history and having visible lesions at slaughter or confirmed bTB. However reactor animals that had a history of being an IC were found to have an increased risk of having visible lesions at slaughter. These animals may actually therefore have been missed positives as the SICCT is imperfect (Monaghan *et al.*, 2004). The delay in detection has given the animal time to develop visible lesions. This is in line with findings by Rodgers *et al.* (2007) who found that increasing time from infection to slaughter resulted in more extensive pathology on post-mortem examination.

Reactors from restricted herds showed significantly less visible lesions at post-mortem and confirmed bTB compared to reactors from routine and at risk tests. This is likely related to the interpretation of the SICCT. In restricted herds, this interpretation is often severe instead of standard. With the interpretation being severe, and therefore having a lower cut off point, more animals are deemed to be reactors and are also detected at an earlier stage of infection, before VL can develop. This finding is in line with Neill *et al*. (1992) who stated that bTB infection is confirmed at post mortem in 66% of the reactors using standard interpretation at the tuberculin skin test compared to 48% for the reactors after severe interpretation. The same effect may be seen in the total number of reactors at the disclosure test. As the test interpretation changes from standard to severe, more animals in the herd will be reactors based on the SICCT test.

In a previous review by Skuce *et al.* (2011) the most commonly identified risk factors for bTB breakdown were cattle movement, occurrence of bTB on contiguous premises and/or in the surrounding areas, herd size, concurrent disease(s), host genetic variation, immune suppression, age and cattle behaviour. Not all of these risk factors have been addressed within the current study in order to compare these with the risk factors identified in the current study for developing visible lesions or bTB confirmation. However of the risk factors examined, risk factors for bTB breakdown seem to be inconsistent with risk factors for developing visible lesions in most cases. For example increasing age is a risk factor for bTB breakdown (Skuce *et al.*, 2011), but similarly it is protective in relation to the development of visible lesions. This would imply that there are different mechanisms underlying to the risk of becoming infected compared to the risk of actually developing visible lesions. There is strong evidence from experimental disease models to link the severity of lesion development with the infectious dose administered to cattle (Pollock *et al.,* 2006). Infection doses of 10^8 and 10^7 colony forming units (CFU) administered subcutaneously induced generalised, systemic tuberculosis untypical of field cases of bTB (Francis, 1958; Waddington and Ellwood, 1972). In contrast, in-contact transmission between experimentally *M. bovis* infected and naive cattle resulted in lesion development typically seen in field pathology (Costello *et al.*, 1998). In addition, Buddle *et al.* (1994) stated the cattle infected with a high dose of *M. bovis* (5×10^5 CFU) had more extensive and more wide spread and larger lesions on post-mortem compared to cattle infected with a low dose of *M. bovis* (5×10^2 CFU). Therefore it is very likely that with respect to the present study, disclosure of visible lesions reflect a stronger infection dose, sufficient to initiate granuloma development. From a practical view point it is of interest which of these risk factors gives scope in relation to the ability of identification of infected animals. Once we are aware which risk factors are protective (such as increased age) for the purpose of abattoir surveillance more vigilance during the post-mortem examination for animals in these categories could be proposed. Further research is needed into lesion development in cattle especially in relation to their immune response to infection in order to be able to understand the findings in the current study more thoroughly. In addition further research is recommended to evaluate the findings of the current study at herd level.

Conclusion

The objective of this study was to get a better insight into the risk factors that affect the development of lesions or positive laboratory tests in bovine tuberculosis reactor cattle in Northern Ireland. Results from this study indicated that a relatively small percentage of reactor animals had visible lesions after post-mortem inspection and/or confirmed bTB by mycobacterial culture. The visible lesion status and bTB status of an animal can affect the way in which bTB breakdowns are managed, since failure to detect visible lesions and recovery of *M. bovis* can lead to a less stringent follow-up after other risk factors have been taken into account. Significant risk factors that increased the risk of visible lesions and/or confirmed bTB in reactor animals were: a diagnosis made in 1998 (rising bTB incidence), large net bovine skin reaction, a history of being inconclusive at a previous test and increasing number of reactors at the disclosure test. Risk factors that decreased the risk of visible lesions and confirmed bTB were increasing age at time of death, dairy breeds, breeding bulls, being disclosed at a restricted test and increasing herd size of the disclosing herd. These risk factors appear to be related to other factors including the susceptibility and immunological status of the animal.

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References

Abernethy, D.A, Pfeiffer, D.U. and Neill, S.D. (2000): Case control study examining the role of livestock markets in the transmission of bovine tuberculosis. Trushfield, M.V., and Goodall, E.A. (Eds.), Society for Veterinary Epidemiology and Preventative Medicine Proceedings, Edinburgh, 29-31 March 2000, 108-112.

Abernethy, D.A., Denny, G.O., Menzies, F.D., McGuckian, P., Honhold, N. and Roberts, A.R. (2006): The Northern Ireland programme for the control and eradication of Mycobacterium bovis. Veterinary Microbiology 112, 1-237.

Abernethy, D. A., Walton, E., Menzies, F., Courcier, E. and Robinson, P. (2011): *Mycobacterium bovis* surveillance in European badgers (*Meles meles*) killed by vehicles in Northern Ireland: an epidemiological evaluation. International Conference on Animal Health Surveillance (ICAHS), Lyon, France, 17-20 May, 2011. (No. 59/60, 216-218). Association pour l'Étude de l'Épidémiologie des Maladies Animales.

Aldridge, B.M., McGuirk, S.M. and Lunn, D.P. (1996): Effect of colostral ingestion on immunoglobulin-positive cells in calves. Veterinary Immunology and Immunopathology 62, 51-64.

Alvarez, J. Perez, A.M., Bezos, J., Casal, C., Romero, B., Roderiquez-Campos, S., Saez-Llorente, J.L., Diaz, R., Carpintero, J., de Juan, L. and Dominiquez, L. (2012): Eradication of bovine tuberculosis at a herd-level in Madrid, Spain: study of within herd transmission dynamics over a 12 year period. BMC Vet Res 8:100. doi 10.1186/1746-6148-8-100.

Anonymous (2011): Northern Ireland Assembly Committee for Agriculture and Rural Development Official Report (Hansard). Policy and Legislation 7 June 2011. On line available at: http://archive.niassembly.gov.uk/record/committees2011/Agriculture/110607PolicyandLegislation.pdf. Accessed 29 July 2013.

Barrington, G.M. and Parish, S.M. (2001): Bovine neonatal immunology. Veterinary Clinical American Food Animal Practice 17(3), 463-476.

Bermingham, M.L., More, S.J., Good, M., Cromie, A.R., Higgins, I.M., Brotherstone, S. and Berry, D.P. (2009): Genetics of Tuberculosis in Irish Holstein-Friesian Dairy Herds, Journal of Dairy Science 92, July 2009, 3447-3456.

Buddle, B.M., Aldwell, F.E., Pfeffer, A, de Lisle, G.W. and Corner, L.A. (1994): Experimental *Mycobacterium bovis* infection of cattle: Effect of dose of *M .bovis* and pregnancy on immune responses and distribution of lesions. New Zealand Veterinary Journal 42, 167-172.

Carrique-Mas, J.J., Medley, G. and Green, L.E. (2008): Risks for bovine tuberculosis in British cattle farms restocked after the foot and mouth disease epidemic of 2001. Preventative Veterinary Medicine 84, 85-93.

Cassidy, J.P., Bryson, D. G., Pollock, J. M., Evans, R. T., Forster, F. and Neill, S. D. (1998): Early lesion formation in cattle experimentally infected with M*ycobacterium bovis*. Journal of Comparative Pathology 119, 27-44.

Corner, L.A. (1994): Post mortem diagnosis of *Mycobacterium bovis* infection in cattle. Veterinary Microbiology 40, 53-63.

Costello, E., Doherty, M.L., Monaghan, M.L., Quigley, F.C. and O'Reilly, P.F. (1998): A study of cattle-to-cattle transmission of *Mycobacterium bovis* infection. The Veterinary Journal 155, 245-250.

DARD (2011): Department of Agriculture and Rural Development, Policy and Economics Division. The Agricultural Census in Northern Ireland, June 2011. Online available at: http://www.dardni.gov.uk/agric-census-in-ni-2011.pdf. Accessed 29 July 2013.

DEFRA (2005): Department of Environment, Food and Rural Affairs. SE3013: Pathogenesis and diagnosis of tuberculosis in cattle-complementary field studies. Online available at: http://randd.defra.gov.uk/Default.aspx?Menu=Menu&Module=More&Location=None&ProjectID=9317. Accessed 29 July 2013.

DEFRA (2007): Department of Environment, Food and Rural Affairs; Independent Scientific Group on cattle TB; Bovine TB: the scientific evidence. Online available at: http://www.defra.gov.uk/aniamlh/tb/isg/final_report.pdf . Accessed 29 July 2013.

DEFRA (2009): Department of Environment, Food and Rural Affairs, Bovine TB- the facts. Online available at: http://archive.defra.gov.uk/foodfarm/farmanimal/diseases/atoz/tb/documents/tb-facts.pdf. Accessed 29 July 2013.

De la Rua-Domenech, R., Goodchild, A.T., Vordermeier, H.M., Hewinson, R.G., Christiansen and Clifton-Hadley, R.S. (2006): Ante mortem diagnosis of tuberculosis in cattle: A review of the tuberculin tests, y-interferon assay and other ancillary diagnostic techniques. Research in Veterinary Science 81, 190-210.

Denny, G.O. and Wilesmith, J.W. (1999): Bovine tuberculosis in Northern Ireland; a case control study of herd risk factors. Veterinary Record 144, 245-250.

Driscoll, E. E., Hoffman, J.I., Green, L.E., Medley, G.F. and Amos, W. (2011): A Preliminary Study of Genetic Factors That Influence Susceptibility to Bovine Tuberculosis in the British Cattle Herd. PLoS ONE 6(4) 2011 Apr 12;6(4):e18806. doi: 10.1371/journal.pone.0018806.

Francis, J., 1958, Tuberculosis in Animals and Man. A study in comparative pathology.

Frankena, K., White, P.W., O'Keeffe, J., Costello, E., Martin, S.W., van Grevenhof, I. and More, S.J. (2007): Quantification of the relative efficiency of factory surveillance in the disclosure of tuberculosis lesions in attested Irish cattle. Veterinary Record 161, 679-684.

Gilbert, M., Mitchell, A., Bourne, D., Mawdsley, J., Clifton-Hadley, R. and Wint, W. (2005): Cattle movements and bovine tuberculosis in Great Britain. Nature 435, 491-496.

Good, M. and Duignan, A. (2011): Perspectives on the History of Bovine TB and the Role of Tuberculin in Bovine TB Eradication. Veterinary Medicine International, 2011, Article ID 410470

Green, L.E. and Cornell, S.J. (2005): Investigations of cattle herd breakdowns with bovine tuberculosis in four counties in England and Wales using VETNET data. Preventative Veterinary Medicine 70, 293-311.

Griffin, J.M., Martin, S.W., Thorburn, M.A., Eves, J.A. and Hammond, R.F. (1996): A case-control study on the association of selected risk factors with the occurrence of bovine tuberculosis in the Republic of Ireland. Preventative Veterinary Medicine 27, 217-229.

Grosset, J. (2003): *Mycobacterium tuberculosis* in the Extracellular Compartment: an Underestimated Adversary. Antimicrobial Agents and Chemotherapy, March 2003, 833-836.

Houston, R. (2001): A computerised database system for bovine traceability. Revenue Scientifique et Technique, International Office of Epizootics, August 2001, 20, 652-661.

Landis, J.R. and Koch, G.G. (1977): The measurement of observer agreement for categorical data. Biometrics 33, 159-174.

McNair, J., Welsh, M. and Pollock, J.M. (2006): Immune responses in bovine tuberculosis: progress toward improved disease control strategies. 4th International Veterinary Vaccines and Diagnostic Conference, Oslo, Norway, 2006

Miranda, M.S., Breiman, A., Allian, S., Deknuydt, F. and Altare, F. (2012): The Tuberculous Granuloma: An Unsuccessful Host Defence Mechanism Providing a Safety Shelter for the Bacteria? Clinical and Developmental Immunology Volume 2012, article ID 139127. Doi:10,1155/2012/139127.

Monaghan, M.L., Doherty, M.L., Collins, J.D., Kazda, J.F. and Quinn, P.J. (2004): The tuberculin test. Veterinary Microbiology 40, 111-124.

Morris, R.S., Pfeiffer, D.U. and Jackson, R. (1994): The epidemiology of *Mycobacterium bovis* infections. Veterinary Microbiology 40, 153-177.

Murray, D., Clegg, T.A. and More, S.J. (2012): Evaluation of single reactor bovine tuberculosis breakdowns based on analysis of reactors slaughtered at an Irish export meat plant. Veterinary Record 170 (20): 516. Epub 2012 Apr 25.

Neill, S.D., Hanna, J., Mackie, D.P. and Bryson, D.G. (1992): Isolation of *Mycobacterium bovis* from the respiratory tract of skin test negative cattle. Veterinary Record 131, 45-47.

OIE (2009): World Organization for Animal Health, OIE Terrestrial Manual 2009. Chapter 2.4.7 Bovine Tuberculosis Online available on: http://www.oie.int. Accessed 29 July 2013.

Olea-Popelka, F.J., White, P.W., Collins, J.D., O'Keeffe J., Kelton, D.F. and Martin, S.W. (2004): Breakdown severity during a bovine tuberculosis episode as a predictor of future herd breakdowns in Ireland. Preventative Veterinary Medicine 63, 163-172.

Olea-Popelka, F.J., Costello, E., White, P., McGrath, G., Collins, J.D., O'Keefe, J. Kelton, D.F., Berke, O., More, S. and Martin, S.W. (2008): Risk factors for disclosure of additional tuberculous cattle in attested-clear herds that had one animal with confirmed lesions of tuberculosis at slaughter during 2003 in Ireland. Preventative Veterinary Medicine 85, 81-91.

Olea-Popelka, F.J., Freeman, Z., White, P., Costello, E., O'Keeffe, J. Frankena, K, Martin, W. and More, S. (2012): Relative effectiveness of Irish factories in the surveillance of slaughtered cattle for visible lesions of tuberculosis, 2005-2007. Irish Veterinary Journal 2012, 65:2.

Palmer, M.V., Whipple, D.L., Rhyan, J.C., Bolin, C.A. and Saari, D.A. (1999): Granuloma development in cattle after intratonsular inoculation with *Mycobacterium bovis*. American Journal of Veterinary Research 60, 310-315

Pollock, J.M., Rodgers, J.D., Welsh, M.D. and McNair, J. (2006): Pathogenesis of bovine tuberculosis: The role of experimental models of infection. Veterinary Microbiology 112, 141-150.

Quinn, P.J., Carter, M.E, Markey, B. and Carter, G.R. (2004): Clinical Veterinary Microbiology. Elsevier Ltd., London, 156.

Ramirez-Villaescusa, A.M., Medley, G.F., Mason, S. and Green, L.E. (2009): Herd and individual animal risks associated with bovine tuberculosis skin test positivity in cattle in herds in south west England. Preventative Veterinary Medicine 92, 188-198.

Ramirez-Villaescusa, A.M., Medley, G.F., Mason, S. and Green, L.E. (2010): Risk factors for herd breakdown with bovine tuberculosis in 148 cattle herds in south west of England. Preventative Veterinary Medicine 95, 224-230.

Rodgers, J.D., Connery, N.L., McNair, J., Welsh, M.D., Skuce, R.A., Bryson, D.G., McMurray, D.N. and Pollock, J.M. (2007): Experimental exposure of cattle to a precise aerosolised challenge of *Mycobacterium bovis*: A novel model to study bovine tuberculosis. Tuberculosis 87, 405-414.

Shittu, A., Clifton-Hadley, R.S., Ely, E.R., Upton, P.U. and Downs, S.H. (2013): Factors associated with bovine tuberculosis confirmation rates in suspect lesions found in cattle at routine slaughter in Great Britain, 2003-2008. Preventative Veterinary Medicine 110, 395-404

Skuce, R.A., Allen, A.R. and McDowell, S.W.J. (2011): Bovine Tuberculosis (TB) A review of cattle-to-cattle transmission, risk factors and susceptibility. Online available on: http://www.dardni.gov.uk/afbi-literature-review-tb-review-cattle-to-cattle-transmission.pdf . Accessed 29 July 2013

Tanaka, S., Tsukada, J., Suzuki, W., Hayashi, K., Tanigaki, K., Tsuji, M., Inoue, H., Honjo, T. and Kubo, M. (2006): The Interleukin-4 Enhancer CNS-2 Is Regulated by Notch Signals and Controls Initial Expression in NKT Cells and Memory-Type CD4 T Cells. Immunity 24, 689-701.

Tanaka, S., Miyazawa, K., Kuwano, A., Watanabe, K., Ohwada, S., Aso, H., Nishida, S. and Yamaguchi, T. (2008): Age-related changes in leucocytes and T cell subsets in peripheral blood of Japanese Black cattle. Animal Science Journal 79, 368-374.

Waddington, F.G. and Ellwood, D.C. (1972): An experiment to challenge the resistance to tuberculosis in BCG vaccinated cattle in Malawi. British Veterinary Journal 128, 541-552.

Whipple, D.L., Bolin, C.A. and Miller, J.M. (1996): Distribution of lesions in cattle infected with *Mycobacterium bovis.* Journal of Veterinary Diagnostic Investigation 8, 351-354.

Wilson, R.A., Zolnai, A., Rudas, P. And Frenyo, L.V. (1996): T-Cell subsets in blood and lymphoid tissues obtained from fetal calves, maturing calves and adult bovine. Veterinary Immunology and Immunopathology 53, 49-60.

Wright, D.M., Allen, A.R., Mallon, T.R., McDowell, S.W.J., Bishop, S.C., Glass, E.J., Bermingham, M.L., Woolliams, J.A. and Skuce, R.A. (2013): Detectability of bovine TB using the tuberculin sin test does not vary significantly according to pathogen genotype within Northern Ireland. Infection, Genetics and Evolution 19, 15-22.

Chapter 3

Farmers' beliefs about bovine tuberculosis control in Northern Ireland

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Abstract

Beliefs can play an important role in farmer behaviour and willingness to adopt new policies. In Northern Ireland, bovine tuberculosis (bTB) is one of the most important endemic diseases facing the cattle industry. An observational study was conducted on 192 farms in a high bTB incidence area during 2010-2011 in order to obtain a better understanding of farmers' beliefs in relation to bTB control. The views of farmers who had experienced a recent confirmed or multiple reactor bTB breakdowns (cases) were compared to those of farmers who had no recent reactors or restricted herd tests (controls). Data were obtained from a face-to-face questionnaire assessing farmers' agreement to 22 statements.

All participating farmers found bTB control important and most were keen to learn more about bTB biosecurity measures and were in favour of the cattle-related bTB control measures as presented in the questionnaire (isolation of skin test inconclusive animals, use of the gamma-interferon test and pre-movement testing). The majority of farmers would allow badger vaccination and culling on their own land with an overall preference for vaccination. Highest disagreement was shown for the statements querying a willingness to pay for bTB control measures. There was agreement on most issues between case and control farmers and between different age groups of farmers although case farmers showed more support for additional advice on bTB biosecurity measures (*P*=0.042). Case farmers were also more in favour of allowing badger vaccination (*P*=0.008) and culling (*P*=0.043) on their land and showed less concern for public opposition (*P*=0.048).

Keywords: Beliefs; Bovine tuberculosis; Case control study; Cattle; Disease control

Introduction

Bovine tuberculosis (bTB), caused by *Mycobacterium bovis*, is a chronic disease with a wide variety of hosts including humans, cattle, sheep, goats, deer, badgers and possums (Pollock and Neill, 2002). It is one of the most important endemic diseases currently facing Government, the veterinary profession and the farming industry in both the United Kingdom and Ireland. The control programme for bTB in Northern Ireland has significant financial consequences with an estimated cost of £23 million[1] in 2010/2011[2] (Anonymous, 2011).

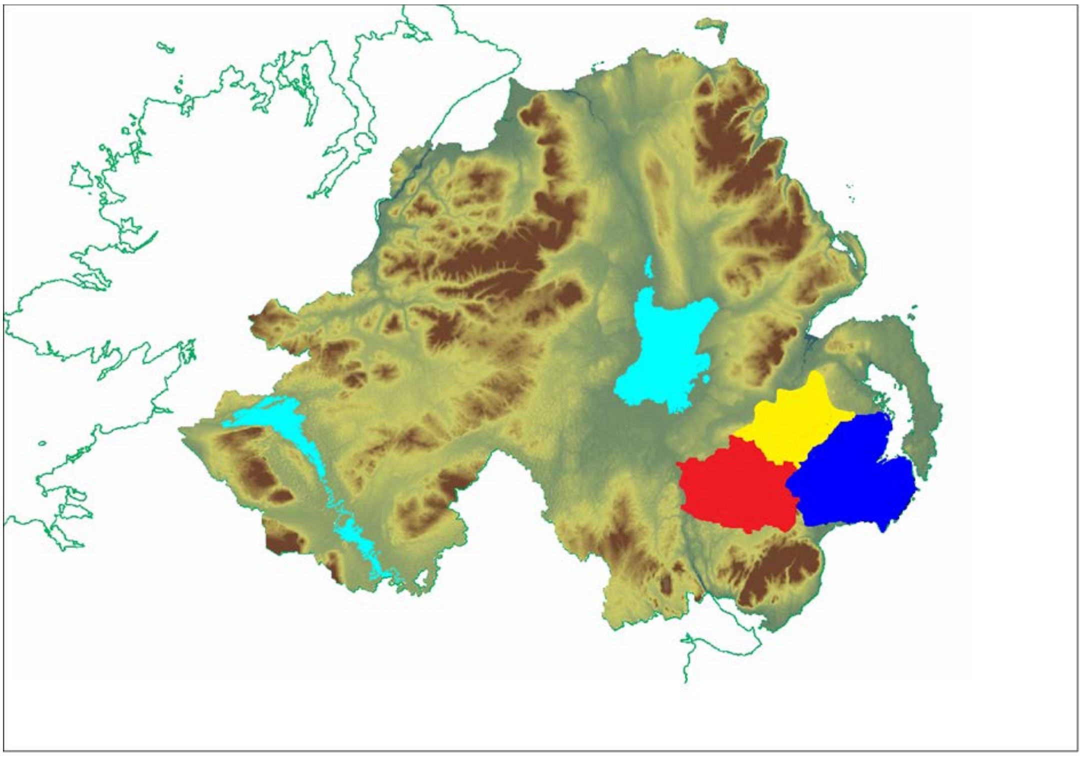
The historic existence of small fragmented farms, the strong reliance on rented pasture, the high level of cattle movement between and within herds and an infectious reservoir in the Eurasian badger (*Meles meles*) are believed to contribute to the maintenance and spread of bTB in Northern Ireland. Current bTB control measures in Northern Ireland are based on annual tuberculin testing using the approved single intra-dermal comparative cervical tuberculin skin test (SICCT). Animals found to be positive are slaughtered and a post-mortem examination is carried out to look for visible lesions and to culture *M. bovis* (Abernethy et al., 2006). A retrospective observational case-control study was undertaken to address the role of possible biosecurity practices in Northern Ireland alongside known cattle-related and badger-related risk factors in relation to bTB. Part of this study, which is reported here, specifically evaluated the beliefs of farmers about bTB and control of the infection. Decisions made by farmers, which are influenced by their attitudes and beliefs, can be important in relation to adoption of new policies and are therefore of interest to Government (Edward-Jones, 2006; Collier et al., 2010). Particular attention was paid to any differences in views between farmers who had experienced a recent bTB breakdown in their herd and those with herds with no recent bTB breakdown history, and also between various age groups of farmers as age can be a significant factor in farmers' attitudes to animal disease management (BVA, 2005; Ellis-Iversen et al., 2011; Sayers et al., 2013; Toma et al., 2013).

[1] £1=approx. US$1.62, €1.25 at 8 September 2014
[2] See: http://www.niassembly.gov.uk/Documents/Official-Reports/Agriculture/2011-2012/PolicyandLegislation.pdf (accessed 9 September 2014)

Materials and methods

A retrospective, observational case-control study was conducted from November 2010 to June 2011. Three Divisional Veterinary Office (DVO) areas in County Down, Northern Ireland with high bTB incidence were allocated as the study area (Fig. 1). The overall study population in this area consisted of 2,575 registered herds. After excluding all herds with less than 10 animals, 1,917 herds were left. Case herds were defined as those that had a confirmed bTB breakdown during 2008 and 2009 (either confirmed reactors or confirmed animals that had visible lesions at routine slaughter) or, if unconfirmed, had two or more reactors.

Figure 1: Map of the study area. The three Divisional Veterinary Office areas enrolled into the study are highlighted.



As herd size is a well-recognized risk factor for bTB breakdown (Griffin et al*.,* 1996; Olea-Popelka et al*.*, 2004; Green and Cornell 2005; Reilly and Courtenay, 2007; Carrique-Mas et al*.*, 2008), cases were selected by stratified random sampling based on herd size category and DVO area. Subsequently control herds (herds within the study area with no reactors or restricted herd tests in the period 2007 to 2009) were group matched with the cases by herd size and DVO area.

Participation

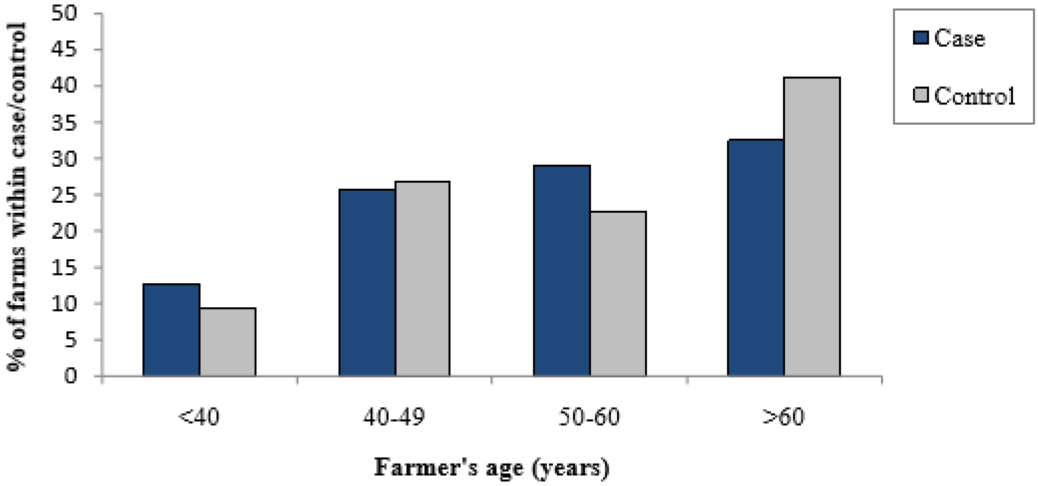
In total 547 invitations to participate were sent to 294 case farms and 253 control farms. Farmers received two phone calls and a reminder letter to stress the importance of their participation in the study.

Seven farms, which were initially selected as control herds, changed to being case herds as they had a confirmed bTB breakdown between the time of selection and the time of the survey. The end result was a participation of 192 farms (117 case herds and 75 control herds); overall participation rate was 35.1% (37.4% for case herds and 32.4% for control herds). In order to assess possible response bias and effects on matching, a comparison between farms that participated and the rest of the study population was conducted by location and herd size. There was no significant difference between study population and participating farmers in relation to location (DVO area), but there was a significant difference in relation to herd size (P=0.016 for cases and P<0.001 for controls).

The farmers' beliefs about bTB and its control were evaluated by means of a face-to-face questionnaire conducted by trained interviewers; the questionnaire was pre-tested using eight farms and amended accordingly. The data collection started in November 2010 and was completed in June 2011. The questionnaire consisted of presenting farmers with 22 statements in relation to bTB control.

The level of agreement to the 22 statements provided was measured using the Likert scale (Likert, 1932). The farmer's age was recorded within four categories (Fig. 2). The questionnaires were entered on a SQL Server database and data were checked for accurate entry.

Figure 2: Age distribution of farmers participating



Statistical analyses were carried out using SPSS Statistics (2010; IBM SPSS Statistics for Windows Version 19.0). To analyse farmers' beliefs, hypothesis testing of differences in level of agreement between case/control and age was carried out using binary logistic regression analyses based on the agreements/non-agreements to the presented statements. In order to complete this, farmers' responses were dichotomized into two categories 'agree' and 'disagree'. These analyses were adjusted for herd size based on the back ground analysis showing a significant difference in herd size between participating farms and farms in the study population. Significance was set at the 5% level.

Principal component analysis was performed on the data, but there were too many components to be meaningful.

Results

Results summary

The detailed results of the responses to the questionnaire are summarised in Table 1 and Table 2. All farmers considered bTB control to be 'important' to 'very important' (Q22).

Table 1: Farmer beliefs about bovine tuberculosis (bTB) and its control. Responses to the questionnaire

Attitude question		Case *n*=117		Control *n*=75		Total *n*=192		Cases vs. Control (Sign.)*
		n	%	n	%	n	%	
(Q1) I would support the introduction of pre-movement TB testing for cattle.	Strongly Agree	5	4.3%	5	6.7%	10	5.2%	
	Agree	59	50.4%	32	42.7%	91	47.4%	
	Neither	6	5.1%	5	6.7%	11	5.7%	
	Disagree	37	31.6%	29	38.7%	66	34.4%	
	Strongly Disagree	10	8.5%	4	5.3%	14	7.3%	
	*Mean**	*1.90*		*1.93*		*1.91*		0.559
(Q2) I would be prepared to pay for pre-movement TB testing.	Strongly Agree	0	0.0%	0	0.0%	0	0.0%	
	Agree	20	17.1%	13	17.3%	33	17.2%	
	Neither	6	5.1%	3	4.0%	9	4.7%	
	Disagree	51	43.6%	37	49.3%	88	45.8%	
	Strongly Disagree	40	34.2%	22	29.3%	62	32.3%	
	*Mean**	*2.95*		*2.91*		*2.93*		0.980
(Q3) I feel that current TB controls on cattle are adequate.	Strongly Agree	4	3.4%	1	1.3%	5	2.6%	
	Agree	69	59.0%	45	60.0%	114	59.4%	
	Neither	8	6.8%	6	8.0%	14	7.3%	
	Disagree	32	27.4%	20	26.7%	52	27.1%	
	Strongly Disagree	4	3.4%	3	4.0%	7	3.6%	
	*Mean**	*1.68*		*1.72*		*1.70*		0.785
(Q4) I feel that it is essential to isolate animals that test inconclusive to a TB test until they‘re retested.	Strongly Agree	11	9.4%	5	6.7%	16	8.3%	
	Agree	80	68.4%	58	77.3%	138	71.9%	
	Neither	5	4.3%	0	0.0%	5	2.6%	
	Disagree	20	17.1%	11	14.7%	31	16.1%	
	Strongly Disagree	1	1.0%	1	1.3%	2	1.0%	
	*Mean**	*1.32*		*1.27*		*1.30*		0.459

Table 1: Continued

Attitude question		Case *n*=117 n	%	Control *n*=75 n	%	Total *n*=192 n	%	Cases vs. Control (Sign.)*
(Q5) I would support greater use of the gamma interferon (blood) test for TB	Strongly Agree	6	5.1%	4	5.3%	10	5.2%	
	Agree	71	60.7%	43	57.3%	114	59.4%	
	Neither	18	15.4%	23	30.7%	41	21.4%	
	Disagree	20	17.1%	5	6.7%	25	13.0%	
	Strongly Disagree	2	1.7%	0	0.0%	2	1.0%	
	*Mean**	*1.50*		*1.39*		*1.45*		0.373
(Q6) I feel that the biosecurity measures that I take will affect my risk of getting TB.	Strongly Agree	2	1.7%	1	1.3%	3	1.6%	
	Agree	74	63.2%	53	70.7%	127	66.1%	
	Neither	11	9.4%	6	8.0%	17	8.9%	
	Disagree	30	25.6%	14	18.7%	44	22.9%	
	Strongly Disagree	0	0.0%	1	1.3%	1	0.5%	
	*Mean**	*1.59*		*1.48*		*1.55*		0.381
(Q7) I feel I have enough information to help me control TB in my herd.	Strongly Agree	1	0.9%	2	2.7%	3	1.6%	
	Agree	95	81.2%	59	78.7%	154	80.2%	
	Neither	4	3.4%	11	14.7%	15	7.8%	
	Disagree	17	14.5%	3	4.0%	20	10.4%	
	Strongly Disagree	0	0.0%	0	1.3%	0	0.5%	
	*Mean**	*1.32*		*1.20*		*1.27*		0.607
(Q8) I would welcome additional advice on measures to prevent TB in my herd.	Strongly Agree	3	2.7%	1	1.4%	4	2.2%	
	Agree	68	61.3%	34	46.6%	102	55.4%	
	Neither	21	18.9%	19	26.0%	40	21.7%	
	Disagree	19	17.1%	17	23.3%	36	19.6%	
	Strongly Disagree	0	0.0%	2	2.7%	2	1.1%	
	*Mean**	*1.50*		*1.79*		*1.62*		**0.042***

Table 1: Continued

Attitude question		Case *n*=117 n	%	Control *n*=75 n	%	Total *n*=192 n	%	Cases vs. Control (Sign.)*
(Q9) I would welcome an advisory visit on biosecurity measures to prevent TB.	Strongly Agree	2	1.7%	0	0.0%	2	1.0%	
	Agree	49	41.9%	36	48.0%	85	44.3%	
	Neither	5	4.3%	3	4.0%	8	4.2%	
	Disagree	59	50.4%	33	44.0%	92	47.9%	
	Strongly Disagree	2	1.7%	3	4.0%	5	2.6%	
	*Mean**	*2.09*		*2.04*		*2.07*		0.746
(Q10) I would be prepared to take further biosecurity measures on my farm to prevent TB.	Strongly Agree	1	0.9%	0	0.0%	1	0.5%	
	Agree	80	68.4%	50	66.7%	130	67.7%	
	Neither	7	6.0%	7	9.3%	14	7.3%	
	Disagree	29	24.8%	18	24.0%	47	24.5%	
	Strongly Disagree	0	0.0%	0	0.0%	0	0.0%	
	*Mean**	*1.55*		*1.57*		*1.56*		0.735
(Q11) I am opposed to badger culling.	Strongly Agree	2	1.7%	2	2.7%	4	2.1%	
	Agree	23	19.7%	12	16.0%	35	18.2%	
	Neither	22	18.8%	10	13.3%	32	16.7%	
	Disagree	62	53.0%	46	61.3%	108	56.3%	
	Strongly Disagree	8	6.8%	5	6.7%	13	6.8%	
	*Mean**	*2.44*		*2.53*		*2.47*		0.549
(Q12) I would support a targeted cull of badgers in problem areas.	Strongly Agree	13	11.1%	6	8.0%	19	9.9%	
	Agree	88	75.2%	59	78.7%	147	76.6%	
	Neither	6	5.1%	4	5.3%	10	5.2%	
	Disagree	9	7.7%	5	6.7%	14	7.3%	
	Strongly Disagree	1	0.9%	1	1.3%	2	1.0%	
	*Mean**	*1.12*		*1.15*		*1.13*		0.894

Table 1: Continued

Attitude question		**Case** *n*=117 n	%	**Control** *n*=75 n	%	**Total** *n*=192 n	%	**Cases vs. Control (Sign.)***
(Q13) I would support culling of badgers on farms that have suffered a TB breakdown.	Strongly Agree	11	9.4%	8	8.8%	17	8.9%	
	Agree	81	69.2%	59	78.7%	140	72.9%	
	Neither	8	6.8%	2	2.7%	10	5.2%	
	Disagree	15	12.8%	7	9.3%	22	11.5%	
	Strongly Disagree	2	1.7%	1	1.3%	3	1.6%	
	*Mean**	*1.28*		*1.17*		*1.24*		0.099
(Q14) I would support a vaccination programme for badgers in problem areas.	Strongly Agree	9	7.7%	4	4.4%	12	6.3%	
	Agree	92	78.6%	53	70.7%	145	75.5%	
	Neither	3	2.6%	5	6.7%	8	4.2%	
	Disagree	9	7.7%	11	14.7%	20	10.4%	
	Strongly Disagree	4	3.4%	3	4.0%	7	3.6%	
	*Mean**	*1.21*		*1.44*		*1.30*		0.054
(Q15) I would support a vaccination programme for badgers rather than culling.	Strongly Agree	4	3.4%	1	1.3%	5	2.6%	
	Agree	63	53.8%	38	50.7%	101	52.6%	
	Neither	24	20.5%	13	17.3%	37	19.3%	
	Disagree	23	19.7%	21	28.0%	44	22.9%	
	Strongly Disagree	3	2.6%	2	2.2%	5	2.6%	
	*Mean**	*1.64*		*1.80*		*1.70*		0.502
(Q16) I would be concerned about public opposition to culling.	Strongly Agree	1	0.9%	1	1.3%	2	1.0%	
	Agree	40	34.2%	31	41.3%	71	37.0%	
	Neither	5	4.3%	5	6.7%	10	5.2%	
	Disagree	69	59.0%	34	45.3%	103	53.6%	
	Strongly Disagree	2	1.7%	4	5.3%	6	3.1%	
	*Mean**	*2.26*		*2.12*		*2.21*		**0.048***

Table 1: Continued

Attitude question		Case *n*=117		Control *n*=75		Total *n*=192		Cases vs. Control (Sign.)*
		n	%	n	%	n	%	
(Q17) I feel that culling badgers would damage the public image of Northern Ireland agriculture.	Strongly Agree	2	1.7%	0	0.0%	2	1.0%	
	Agree	36	30.8%	30	40.0%	66	34.4%	
	Neither	9	7.7%	6	8.0%	15	7.8%	
	Disagree	63	53.8%	34	45.3%	97	50.5%	
	Strongly Disagree	7	6.0%	5	6.7%	12	6.3%	
	*Mean**	*2.32*		*2.19*		*2.27*		0.247
(Q18) I would allow badgers to be culled on my land.	Strongly Agree	5	4.3%	5	6.7%	10	5.2%	
	Agree	94	80.3%	48	64.0%	142	74.0%	
	Neither	8	6.8%	8	10.7%	16	8.3%	
	Disagree	10	8.5%	14	18.7%	24	12.5%	
	Strongly Disagree	0	0.0%	0	0.0%	0	0.0%	
	*Mean**	*1.20*		*1.41*		*1.28*		**0.043***
(Q19) I would allow badgers on my land to be vaccinated.	Strongly Agree	7	6.0%	1	1.3%	8	4.2%	
	Agree	103	88.0%	61	81.3%	164	85.4%	
	Neither	0	0.0%	3	4.0%	3	1.6%	
	Disagree	7	6.0%	10	13.3%	17	8.9%	
	Strongly Disagree	0	0.0%	0	0.0%	0	0.0%	
	*Mean**	*1.06*		*1.29*		*1.15*		**0.008****
(Q20) I would be prepared to pay for badgers on my land to be culled.	Strongly Agree	0	0.0%	0	0.0%	0	0.0%	
	Agree	27	23.1%	12	16.0%	39	20.3%	
	Neither	8	6.8%	7	9.3%	15	7.8%	
	Disagree	61	52.1%	42	56.0%	103	53.6%	
	Strongly Disagree	21	17.9%	14	18.7%	35	18.2%	
	*Mean**	*2.65*		*2.77*		*2.70*		0.425

Table 1: Continued

Attitude question		**Case** *n*=117 n	%	**Control** *n*=75 n	%	**Total** *n*=192 n	%	**Cases vs. Control (Sign.)***
(Q21) I would be prepared to pay for badgers on my land to be vaccinated.	Strongly Agree	0	0.0%	0	0.0%	0	0.0%	
	Agree	27	23.1%	9	12.0%	36	18.8%	
	Neither	10	8.5%	9	12.0%	19	9.9%	
	Disagree	65	55.6%	48	64.0%	113	58.9%	
	Strongly Disagree	15	12.8%	9	12.0%	24	12.5%	
	*Mean**	*2.58*		*2.76*		*2.65*		0.056
(Q22) Overall how important is the control of TB to you?	Very Important	99	84.6%	57	76.0%	156	81.3%	
	Important	18	15.4%	18	24.0%	36	18.8%	
	Not Important	0	0.0%	0	0.0%	0	0.0%	
	*Mean**	*0.15*		*0.24*		*0.19*		***

- Case vs. control (Sign.)*: the value shown is the likelihood ratio significance based on agreements/non-agreements with the statement for the cases and controls. Analysis conducted is binary logistic regression modelling adjusted for herd size and age group.
- Mean*: the value shown is the mean response from the ordered score indicating the overall strength of agreement (5 point scale: Strongly agree=0, Strongly disagree=4)

* $P<0.05$

** $P<0.01$

*** No likelihood ratio significance was available for this statement as all participating farmers found TB control important

Table 2: Farmer beliefs about bTB and its control. Mean rank of attitude scores by farmers' age category

Attitude question	Case (*n*=117)/ Control (*n*=75)	Residual ranks* by Farmer's age category*				Age Sign.*
		<40 years	**40-49 years**	**50-60 years**	**>60 years**	
(Q1) I would support the introduction of pre-movement TB testing for cattle.	Case	-8.00	6.52	-4.04	1.63	0.181
	Control	-1.57	-4.00	-6.06	6.26	
(Q2) I would be prepared to pay for pre-movement TB testing.	Case	1.00	7.48	-7.50	0.41	0.261
	Control	1.43	-2.22	2.47	-0.24	
(Q3) I feel that current TB controls on cattle are adequate.	Case	-6.70	1.38	-1.12	2.55	0.655
	Control	-3.00	5.88	-1.94	-2.05	
(Q4) I feel that it is essential to isolate animals that test inconclusive to a TB test until they are retested.	Case	0.33	-2.67	1.06	1.03	0.535
	Control	-3.50	5.43	2.76	-4.23	
(Q5) I would support greater use of the gamma interferon (blood) test for TB	Case	6.30	-4.43	3.53	-2.14	**0.047***
	Control	-7.29	-2.58	**12.12**	-3.34	
(Q6) I feel that the biosecurity measures that I take will affect my risk of getting TB.	Case	-4.07	-3.23	2.78	1.67	0.876
	Control	1.29	1.48	-1.44	-0.45	
(Q7) I feel I have enough information to help me control TB in my herd.	Case	-2.00	-0.70	5.57	-3.64	0.065
	Control	-1.00	0.17	4.97	-2.61	
(Q8) I would welcome additional advice on measures to prevent TB in my herd.	Case	-1.03	0.20	-9.52	-0.76	0.610
	Control	-0.43	1.08	6.62	-6.77	
(Q9) I would welcome an advisory visit on biosecurity measures to prevent TB.	Case	-8.30	0.42	-3.44	6.03	0.912
	Control	9.86	0.23	2.56	-3.77	
(Q10) I would be prepared to take further biosecurity measures on my farm to prevent TB.	Case	-9.3	4.55	-4.62	4.21	0.221
	Control	-6.64	0.02	1.97	0.44	
(Q11) I am opposed to badger culling.	Case	5.67	0.30	0.13	-2.86	0.598
	Control	9.50	-0.67	-7.26	2.27	
(Q12) I would support a targeted cull of badgers in problem areas.	Case	-4.33	-6.77	4.74	2.82	0.943
	Control	2.50	0.15	0.00	-0.66	

Table 2: continued

Attitude question	Case (*n*=117)/ Control (*n*=75)	Residual ranks* by Farmer's age category*				Age Sign.*
		<40 years	**40-49 years**	**50-60 years**	**>60 years**	
(Q13) I would support culling of badgers on farms that have suffered a TB breakdown.	Case	1.80	-5.00	8.71	-4.55	0.289
	Control	3.00	-0.10	-0.06	-0.58	
(Q14) I would support a vaccination program for badgers in problem areas.	Case	-3.10	-2.88	1.24	2.39	0.642
	Control	-3.86	4.90	-0.94	-1.77	
(Q15) I would support a vaccination program for badgers rather than culling.	Case	1.33	3.43	-8.72	4.57	0.108
	Control	-4.14	5.18	-5.82	0.79	
(Q16) I would be concerned about public opposition to culling.	Case	3.17	-3.03	-8.18	8.46	0.387
	Control	-13.07	-2.17	2.09	3.21	
(Q17) I feel that culling badgers would damage the public image of Northern Ireland agriculture.	Case	8.3	-5.08	**-11.66**	**11.96**	**0.034***
	Control	-9.07	0.33	-3.59	3.81	
(Q18) I would allow badgers to be culled on my land.	Case	-6.40	2.65	3.82	-2.99	0.465
	Control	5.07	1.40	0.85	-2.52	
(Q19) I would allow badgers on my land to be vaccinated.	Case	3.67	1.83	-1.62	-1.45	0.826
	Control	6.00	2.98	3.12	-2.27	
(Q20) I would be prepared to pay for badgers on my land to be culled.	Case	-10.20	9.48	-1.93	-1.74	0.341
	Control	-3.21	3.18	0.21	-1.44	
(Q21) I would be prepared to pay for badgers on my land to be vaccinated.	Case	-4.83	**14.18**	-4.71	-5.08	**0.025***
	Control	**-15.64**	5.93	5.12	-3.10	
(Q22) Overall how important is the control of TB to you?	Case	-9.00	6.60	-2.12	0.24	***
	Control	7.07	4.13	-6.79	-0.53	

- Residual ranks*: this refers to the difference between the mean rank and the expected rank (i.e. if there was no effect). The expected rank for cases is 59 (*n*+1/2) and the expected rank for controls is 38 (*n*+1/2). Disagreement with the statement is reflected by a higher score. A positive residual rank refers to the mean rank > expected rank and a negative residual rank refers to the mean rank < expected rank.
- Farmer's age category*: < 40 years (*n*=22); 40-49 years (*n*=50); 50-60 years *(n*=51); >60 years (*n*=69)
- Age (Sign.)*: the value shown is the likelihood ratio significance based on agreements/non-agreements with the statement across the different age categories. . Analysis conducted is binary logistic regression modelling adjusted for herd size and case/control status.

* *P*<0.05

** *P*<0.01

*** No likelihood ratio significance was available for this statement as all participating farmers found TB control important

Most farmers believed that the current bTB controls were ‘adequate’ (62.0%) and that they had enough information to help them control bTB in their herd (81.8%). However, 45.3% of farmers said they would welcome an advisory visit on biosecurity measures to prevent bTB and 57.6% of farmers stated that they would welcome additional advice on measures to prevent bTB in their herd with the difference between cases (64.0%) and controls (48.0%) being significant (adjusted Odds Ratio [OR] = 1.91 (95% Confidence Interval [CI] 1.02-3.57); *P*=0.042).
Biosecurity measures were thought to make a significant difference to the risk of introducing bTB infection (67.7%), which was also reflected in farmers’ preparedness to take further biosecurity measures in the future (68.2%).

Beliefs in relation to cattle related bTB control measures

Most farmers were in agreement with respect to cattle-related bTB control measures, with the majority (80.2%) reporting that they believed it to be essential to isolate animals that were inconclusive to a bTB test until they had been retested. In addition, the majority of farmers stated that they would support greater use of the γ-interferon (IFN) blood test (64.6%) and the introduction of pre-movement bTB testing (52.6%). Interestingly, only 17.2% of farmers said they would be prepared to pay for pre-movement bTB testing.

Beliefs in relation to badger related bTB control measures

Statements were presented on culling and vaccination of badgers and both received good support from the participating farmers although 20.3% said they were opposed to a badger cull. Notably, 86.5% of farmers stated they would support a targeted badger cull in problem areas and 81.8% stated they would be in favour of badger culls on farms that have suffered a bTB breakdown. Most participating farmers (79.2%) stated that they would allow badger culling on their land and the difference between cases (84.6%) and controls (70.7%) was significant (adjusted OR= 2.18 [95% CI 1.03-4.62]; *P*=0.043). However, only 20.3% of farmers said they were willing to pay for it.

Approximately one-third of the farmers (38.0%) were concerned about public opposition to culling with the difference between cases (35.1%) and controls (44.6%) being significant (adjusted OR= 0.51 [95% CI 0.27-0.99]; *P*=0.048) and 35.4% felt that it would damage the public image of Northern Ireland. The answers to the questionnaire reflected that 81.8% of the participating farmers would support a badger vaccination program in problem areas and 89.6% would allow badgers to be vaccinated on their own land with the difference between cases (94.0%) and controls (82.6%) being significant (adjusted OR= 4.34 [95% CI 1.48-12.77]; *P*=0.008). However, only 18.8% of farmers were willing to pay for this suggested bTB control measure. Although the same proportion of farmers (81.8%) supported badger culling and badger vaccination on farms that had experienced a bTB breakdown/problem, the vaccination strategy gained the most support (55.2% vs. 25.5%) when farmers were asked which of the two control measures they would prefer.

Differences in beliefs between cases and controls

In general, there were very few significant differences in beliefs between cases and controls. However, case farmers showed significantly (*P*=0.042) more support for additional advice on measures to prevent bTB in their herd, and they were more concerned about public opposition to culling of badgers (*P*=0.048) and allowing badgers to be culled on their land (*P*=0.043). When asked whether they would allow badgers to be vaccinated on their land, 89.6% of farmers stated that they would with a significant difference (*P*=0.008) in responses between cases and controls.

Differences in beliefs between age groups

The majority of farmers were over 50 years of age (Fig. 2). There were very few differences in beliefs between of farmers in different age groups (see Table 2), although case farmers in the age group 40-49 years and 50-60 years showed a significantly higher level of agreement with the statement that culling badgers would damage the public image of Northern Ireland agriculture (adjusted OR, respectively, 2.86 [95% CI 1.26-6.43] and 2.89 [95% CI 1.28-6.56]) compared to farmers over 60 years old (*P*=0.034). Also farmers in the age group 40-49 years showed a significantly higher level of disagreement compared to the other age groups in relation to their willingness to pay for badgers on their land being vaccinated (adjusted OR 0.14 [95% CI 0.03-0.67]; *P*=0.025). Farmers in the age category 50-60 years showed a significantly lower level of agreement with the statement whether they would support greater use of the γ-IFN test (adjusted OR=0.35 [95% CI 0.16-0.78], *P*=0.047).

Discussion

Attitudes have been defined as ‘*a positive or negative response towards a person, idea, concept or physical object*’ (Willock et al., 1999). Within an agricultural context it has become more apparent amongst agricultural scientists that beliefs and attitudes can play an important role in farmer behaviour and their willingness to adopt new policies (Edward-Jones, 2006). This study aimed to obtain a better understanding of farmers’ beliefs towards bTB control in Northern Ireland. Although limited by the low participation rate (35.1%), which may have caused some bias, the fact that the participation rates were very similar for case herds (37.4%) and control herds (32.4%) gave confidence that the results were likely to be reliable. In addition, background analyses leading to adjustment of the statistical analyses have attempted to address this. Although the study area did not encompass the whole of Northern Ireland, the results from this study should be a good indicator of farmers’ beliefs in the rest of the Province due to similar farming practices and bTB control policies.

It was clear from our study that, in line with findings by Warren et al*.* (2013), the vast majority of farmers believed that the control of bTB is very important and that they were well informed on measures they could take to control bTB in their herd. Nevertheless, farmers were keen to learn more about the control of bTB in the form of additional advice.

The majority of farmers (67.7%) stated that biosecurity measures were thought to make a significant difference to the risk of introducing bTB infection and most farmers (68.2%) were prepared to take further biosecurity measures in the future. This is in line with the findings from Brennan and Christley (2013), who reported that the majority of biosecurity practices prompted to the participating farmers were deemed to be useful.

In the current study, participating farmers generally were in favour of the presented cattle-related bTB control measures (isolation of skin test inconclusive animals, additional use of the γ-IFN test and pre-movement testing). Nevertheless, although pre-movement bTB testing did get support, most farmers said they were unwilling to pay for it. Bennett (2009), who questioned 60 cattle farmers in England on their attitude to pre-movement testing for bTB, also found a resistance mainly based on the cost involved. In addition, he stated that just over half of the farmers did not believe pre-movement testing was effective. Christley et al*.* (2011) reported similar findings after conducting a survey amongst 800 cattle farmers in England and Wales after the introduction of pre-movement testing which resulted in a reduction in movements of cattle between farms in order to avoid pre-movement testing.

Results from our study indicated that there was strong support for both badger culling and badger vaccination from the participating farmers, but that in general they would prefer a badger vaccination programme. However, conflation of beliefs about efficiency of control measures and the responsibility for payment of costs of bTB control measures should be noted as the majority of farmers stated that they would allow a badger cull and badger vaccination on their own land, as long as there was no cost involved for them. These findings are in contrast with previous research conducted by Bennett and Cooke (2005), which consisted of a survey of a random sample of 151 English cattle farmers whose cattle herds had suffered a bTB breakdown. That study suggested that farmers only had a moderate level of support for badger vaccination and that they preferred a culling programme for badgers as a bTB control measure. Enticott et al*.* (2012) found similar results with the majority of farmers in his study being cautious about badger vaccination, appearing to be neither overly confident nor unconfident in it. A recent study by Warren et al*.* (2013) found that 10/14 farmers interviewed in their survey were positive to varying degrees to badger vaccination as a means of bTB control in cattle, but mainly based on 'resigned acceptance'. In the same survey, 50% of farmers thought culling was more effective than vaccination.

Approximately one-third of farmers participating in our study had little concern about public opposition to badger culling. This was in line with White and Whiting (2000), who published results, based on a 100 person questionnaire, stating that an experimental trial of culling badgers was considered to be acceptable. Similarly, a telephone and postal survey by Bennett and Willis (2007) stated that the majority of the public agreed with a limited or temporary cull of badgers if it would solve the bTB problem.

Farmers showed the highest disagreement with the questions which queried their willingness to pay for control measures in relation to bTB. This has been reported by previous studies on several occasions (Bennett and Cooke, 2005; Hovi, 2005; Gunn et al., 2008; Bennett, 2009; Brennan and Christley, 2012). Stott et al. (2005), who conducted a survey of 96 farmers in Scotland, stated that the vast majority (94.7%)

of the participating farmers thought that if disease control was necessary from a public health control perspective, then the Government should finance the control measures. However, farmers in bTB hotspots areas in England and Wales were said to be willing to pay for a bTB cattle vaccine, with most of them prepared to pay even more than the expected cost of such a vaccine (Bennett and Balcombe, 2012). Conflicts over beliefs in relation to responsibility of disease control highlight a potential obstacle in the current disease control programme as, although farmers find bTB control important, they see the Government as financially responsible for the disease control. In Australia, the model of joint industry and government funding and decision-making used during the tuberculosis eradication campaign, was highly successful (Radunz, 2006; More, 2008).

In general, although there were relatively few differences between the beliefs of farmers that had a bTB problem in their herd and farmers that had no recent history of bTB infection on their farm, there were some significant differences suggesting that when farmers had actually experience an outbreak, it can change their beliefs in relation to certain issues. This has also been described in previous studies (Enticott and Vanclay, 2011; Hernandez-Jover et al., 2012; Zingg and Siegrist, 2012).

The majority of farmers participating in our study were over 50 years of age, which is similar to figures described in the European Union Farm Structure Survey[1], which quoted the median age of principal farmers in Northern Ireland to be 57 years in 2010, with 55% of principal farmers being 55 years or older and 20% being 44 years or younger. There were few significant differences detected between the different age groups of participating farmers.

Conclusions

Translation of a better understanding of beliefs and behaviours into policy can influence change. The findings of this study indicate that farmers agree with the importance of bTB control in Northern Ireland and that they have a very positive attitude to bTB control measures regardless of the bTB history of their own herd. However, generally they regard the financial implications of these measures as a problem for themselves regardless of whether or not they had experienced a recent

[1] See: http://www.dardni.gov.uk/european_structure_survey_2010.pdf (accessed 9 September 2014)

bTB breakdown in their herd. Differences and conflicts between attitudes towards a specific disease control measure and attitudes in relation to the responsibility for the actual disease control are highlighted in this study. Greater involvement of industry with regards to funding and decision making in relation bTB control may be able to address these obstacles.

Conflict of interest

None of the authors has any financial or personal relationships that could inappropriately influence or bias the content of the paper.

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References

Abernethy, D.A., Pfeiffer, D.U, Watt, R., Denny, G.O., McCullough, S., McDowell, S.W., 2006. Epidemiology of bovine brucellosis in Northern Ireland between 1990-2000. Veterinary Record 158, 717-721.

Bennett, R.M., Cooke, R.J., 2005. Control of bovine TB: preferences of farmers who have suffered a TB breakdown. Veterinary Record 156, 143-145.

Bennett, R., Willis, K., 2007. Public opinions on badger populations and the control of tuberculosis in cattle in the UK, Veterinary Record 160, 268-269.

Bennett, R.M., 2009. Farm cost associated with pre-movement testing for bovine tuberculosis. Veterinary Record 164, 77-79.

Bennett, R.M., Balcombe, K., 2012. Farmers' willingness to pay for a tuberculosis cattle vaccine. Journal of Agricultural Economics 63, 408-424

Brennan, M.L., Christley, R.M., 2012. Biosecurity on cattle farms: a study in North-West England. PLoS ONE 7(1): e28139.doi:10.1371/journal.pone.0028139

Brennan, M.L., Christley, R.M., 2013. Cattle producers' perceptions of biosecurity. BMC Veterinary Research 9:71. Doi: 10.1186/1746-6149-9-71.

BVA, 2005. Biosecurity: challenges of disease control at every level. Veterinary Record 157, 461-464.

Carrique-Mas, J.J., Medley, G.F., Green, L.E., 2008. Risk of bovine tuberculosis in British cattle farms restocked after the foot and mouth epidemic of 2001. Preventive Veterinary Medicine 84, 85-93.

Christley, R.M., Robinson, S.E., Moore, B., Setzkorn, C., Donald, I., 2011. Responses of farmers to introduction in England and Wales of pre-movement testing for bovine tuberculosis. Preventive Veterinary Medicine 100, 126-133.

Collier, A., Cotterill, A., Everett, T., Muckle, R., Pike, T., Vanstone, A. , 2010. Understanding and influencing behaviours: a review of social research, economics and policy making in Defra. Department for Environment, Food and Rural Affairs, London.

Edward-Jones, G., 2006. Modeling farmer decision making: concept, progress and challenges. Animal Science 82, 783-790.

Ellis-Iversen, J., Cook, A.J.C., Watson, E., Nielen, M., Larkin, L., Wooldridge, M., Hogeveen, H., 2011. Perceptions, circumstances and motivators that influence implementation of zoonotic control programmes on cattle farms. Preventive Veterinary Medicine 93, 276-285.

Enticott, G., Vanclay, F., 2011. Scripts, animal health and biosecurity: The moral accountability of farmers' talk about animal health risks. Health Risk and Society 13, 293-309.

Enticott, G., Maye, D., Ilbery, B., Fisher, R., Kirwan, J., 2012. Farmers' confidence in vaccinating badgers against bovine tuberculosis. Veterinary Record 170, 204, doi:10.1136/vr.100079

Green, L.E., Cornell, S.J., 2005. Investigations of cattle herd breakdowns with bovine tuberculosis in four countries of England and Wales using VetNet data. Preventive Veterinary Medicine 70, 293-311.

Griffin, J.M., Martin, S.W., Thorburn, M.A., Eves, J.A., Hammond, R.F., 1996. A case-control study on the association of selected risk factors with the occurrence of bovine tuberculosis in the Republic of Ireland. Preventive Veterinary Medicine 27, 217-229.

Gunn, G.J., Heffernan, C., Hall, M., McLeod, A., Hovi, M., 2008. Measuring and comparing constraints to improve biosecurity among GB farmers, veterinarians and the auxiliary industries. Preventive Veterinary Medicine 84, 310-323.

Hernandez-Jover, M., Taylor, M., Holyoake, P., Dhand, N., 2012. Pig producers' perceptions of the Influenza Pandemic H1N1/09 outbreak and its effect on their biosecurity practices in Australia. Preventive Veterinary Medicine 106, 284-294.

Hovi, M., McLeod, A., Gunn, G., 2005. Assessing UK farmer attitudes to biosecurity on sheep and cattle farms. Research in Veterinary Science 78: 24

Likert, R., 1932. A Technique for the Measurements of Attitudes. Archives of Psychology 140, 1-55.

More, S.J., 2008. A case for increased private sector involvement in Ireland's national animal health services. Irish Veterinary Journal 61, 70-78.

Olea-Popelka, F.J., White, P.W., Collins, J.D., O'Keeffe, J., Kelton, D.F., Martin, S.W., 2004. Breakdown severity during a bovine tuberculosis episode as a predictor of future herd breakdowns in Ireland. Preventive Veterinary Medicine 63, 163-172.

Pollock, J.M., Neill, S.D., 2002. *Mycobacterium bovis* infection and tuberculosis in cattle. The Veterinary Journal 163, 115-127.

Radunz, B., 2006. Surveillance and risk management during the latter stages of eradication: Experiences from Australia. Veterinary Microbiology 112, 283-290.

Reilly, L.A., Courtenay, O., 2007. Husbandry practices, badger sett density and habitat composition as risk factors for transient and persistent bovine tuberculosis on UK cattle farms. Preventive Veterinary Medicine 80, 129-142.

Sayers, R.G., Sayers, G.P., Mee, J.F., Good, M., Bermingham, M.L., Grant, J., Dillon, P., 2013. Implementing biosecurity measures on dairy farms in Ireland. The Veterinary Journal 197, 259-267.

Stott, A.W., Milne, C.E., Peddie, S., Gunn, G.J., 2005. Balancing public and private interests in EU food production. 56th Annual Meeting European Association for Animal Production (EAAP), Uppsala, Sweden, June 5th - 8th, 2005. Book of abstracts, p. 4.

Toma, L., Stott, A.W., Heffernan, C. Ringrose, S., Gunn, G.J., 2013. Determinants of biosecurity behaviour of British cattle and sheep farmers-A behavioural economics analysis. Preventive Veterinary Medicine 108, 321-333.

Warren, M., Lobley, M., Winter, M., 2013. Farmer attitudes to vaccination and culling of badgers in controlling bovine tuberculosis. Veterinary Record 173:40. doi: 10.1136/vr.101601.

White, P.C.L., Whiting, S.J., 2000. Public attitudes towards badger culling to control bovine tuberculosis in cattle. Veterinary Record 147, 179-184.

Willock, J., Deary, I.J., McGregor, M.J., Sutherland, A., Edwards-Jones, G., Morgan, O., Dent, J.B., Grieve, R., Gibson, G., Austin, E., 1999. Farmers' attitudes, objectives, behaviours and personal traits. The Edinburgh study of decision making on farms, Journal of Vocational Behaviour 54, 5-36.

Zingg, A., Siegrist, M., 2012. Lay people's and experts' risk perception and acceptance of vaccination and culling strategies to fight animal epidemics. Journal of Risk Research 15, 53-66.

Chapter 4

Herd-level risk factors for bovine tuberculosis and adoption of related biosecurity measures in Northern Ireland: A case-control study

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Abstract

Bovine tuberculosis (bTB) is a zoonotic disease which is endemic in Northern Ireland. As it has proven difficult to eradicate this disease, partly due to a wildlife reservoir being present in the European badger (*Meles meles*), a case-control study was conducted in a high incidence area in 2010-2011. The aim was to identify risk factors for bTB breakdown relating to cattle and badgers and to assess the adoption of bTB related biosecurity measures on farms. Face-to-face questionnaires with farmers and surveys of badger setts and farm boundaries were conducted on 117 farms with (cases) and 75 farms without (controls) a recent bTB breakdown. Logistic regression at univariable and multivariable level disclosed significant risk factors associated with being a case herd, including having an accessible badger sett within the farm boundaries in a field grazed in the last year (odds ratio, OR, 4.14; 95% confidence interval, CI, 1.79, 9.55), observation of live badgers (OR 4.14; 95% CI 1.79, 9.55), purchase of beef cattle (OR 4.60; 95% CI 1.61, 13.13), use of contractors to spread slurry (OR 2.83; 95% CI 1.24, 6.49), feeding meal on top of silage (OR 3.55; 95% CI 1.53, 8.23) and feeding magnesium supplement (OR = 3.77; 95% CI 1.39, 10.17). The majority of setts within the farm boundary were stated to be accessible by cattle (77.1%; 95% CI 71.2, 83.0%) and 66.8% (95% CI 63.8, 69.7%) of farm boundaries provided opportunities for nose-to-nose contact between cattle. Adoption of bTB related biosecurity measures, especially with regards to purchasing cattle and badger-related measures, was lower than measures in relation to disinfection and washing.

Keywords: Bovine tuberculosis; *Mycobacterium bovis*; Case-control study; Risk Factors; Biosecurity

Introduction

Bovine tuberculosis (bTB), caused by *Mycobacterium bovis,* is a zoonotic disease which is endemic in many species worldwide (Pollock and Neill, 2002). In Northern Ireland, the disease has consequences for animal and human health, alongside being a financial burden for Government. A control programme based on test-and-slaughter (Council Directive 64/432/EEC) has been in place since the 1950s, but has not lead to eradication, possibly due to fragmented farms, dependence on rented pasture, frequent inter-herd cattle movement and the presence of a wildlife reservoir, the European badger (*Meles meles*) (Abernethy et al., 2006).

Many studies have been conducted assessing risk factors for bTB breakdown, as summarised by Skuce et al. (2012), with cattle movement, bTB outbreaks on neighbouring farms, bTB history and herd size being commonly described herd-level risk factors. Comparison of studies such as these is complicated by differing outcomes due to variation in farm management, farm structure, regional bTB incidence and wildlife density in the study areas. Although previous studies have focused on biosecurity measures mitigating potential risk factors for bTB transmission (Philips et al., 2003; Ward et al., 2010; Johnston et al., 2011; Judge et al., 2011; Wilson et al., 2011), there is as yet no empirical evidence linking improved biosecurity with reduced risk of bTB breakdown. Furthermore, great variation in uptake of biosecurity measures has been reported previously in Great Britain (Brennan and Christley, 2012; Cresswell et al., 2014), Ireland (Sayers et al., 2013) and further afield (Brandt et al., 2008; Nöremark et al., 2010). No such assessment had ever been conducted in Northern Ireland. Biosecurity measures examined in our work are based on previously suggested management ideas (Phillips et al., 2003; Ward et al., 2010) relating to prevention of bTB introduction into the herd by badgers, neighbouring cattle, cattle purchases and indirect transmission.

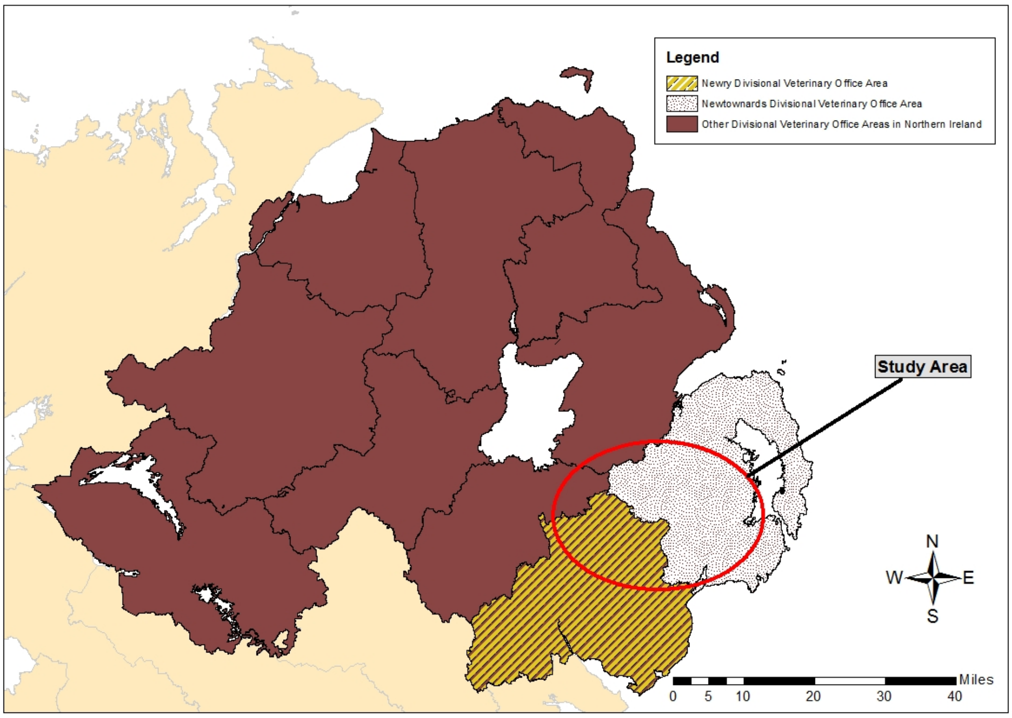
The current study had the following objectives: (1) to assess the level of adoption of biosecurity measures in Northern Ireland; and (2) to assess the impact of potential bTB biosecurity measures on the risk of bTB breakdown. The results in relation to a third objective (assessing farmers' attitudes toward bTB control) have previously been reported in O'Hagan et al. (2016).

Materials and methods

Study area

For logistical reasons and in order to decrease variability, a defined study area was chosen in an area of high incidence. This area consisted of parts (area 27/37/39) of two divisional veterinary office (DVO) areas with an annual bTB herd incidence for 2010 of 4.92% in DVO Newry and 7.86% in DVO Newtownards[1] (Fig 1).

Figure 1: Map of the study area used for a case-control study of 117 farms with a bovine tuberculosis breakdown and 75 farms without a breakdown in Northern Ireland in 2008/2009



Study population

There were 2281 active herds in the study area based on the fact that they were subjected to the single intradermal comparative cervical skin test (SICCT) in 2008 and/or 2009. Inclusion of herds into the study by location was based on point map references of the main farm house since, for practical purposes, this is the best geo-referencing method (Durr and Froggatt, 2002). As the SICCT used to disclose bTB infected animals is not 100% specific (Clegg et al., 2011), case herds were selected

[1] See: https://www.dardni.gov.uk/sites/default/files/publications/dard/tb-stats-december2010.pdf (accessed on 22 March 2016)

on the basis of the presence of confirmed or multiple reactors. The case definition was therefore ‘herds in the study area that during 2008 and/or 2009 had multiple reactors to the SICCT or a confirmed bTB breakdown (based on either one or more confirmed reactors to the SICCT, or one or more confirmed animals that were detected at post-mortem examination during routine slaughter). Confirmation was based on having positive histopathology or bacteriology findings (O’Hagan et al., 2015). The control selection definition was ‘herds in the study area without restricted herd tests or reactors to the SICCT from 2007 to 2009’. Small herds (< 10 animals) were excluded from the study.

Herd size is a known risk factor in relation to bTB breakdown (Griffin et al., 1996; Olea-Popelka et al., 2004; Green and Cornell, 2005; Abernethy et al., 2010). Background analysis assessing the distribution of active herds in the study area by herd size and case-control status confirmed that large herds were more likely to be cases. Therefore, cases were selected by stratified random sampling based on herd size category (see Table 1) and DVO area. Subsequently, controls were selected on a group-matched basis (1:1 match).

Table 1: Distribution of sampled and participating case and control farms by herd size category (case-control study of 117 and 75 farms with and without a bovine tuberculosis breakdown in Northern Ireland in 2008/2009)

Herd size category*	**Number of cases in study population**	**Number of controls in study population**	**Number cases (%) sampled**	**Number controls (%) sampled**	**Number cases (%) participating**	**Number controls (%) participating**
10-19	35 (7.8%)	304 (30.2%)	20 (6.8%)	20 (7.9%)	4 (3.4%)	2 (2.7%)
20-29	27 (6.0%)	180 (17.9%)	20 (6.8%)	20 (7.9%)	4 (3.4%)	6 (8.0%)
30-49	60 (13.4%)	199 (19.8%)	37 (12.6%)	37 (14.6%)	10 (8.5%)	8 (10.7%)
50-99	119 (26.5%)	198 (19.7%)	74 (25.2%)	74 (29.2%)	23 (19.7%)	29 (38.7%)
100-199	103 (22.9%)	95 (9.4%)	64 (21.8%)	72 (28.5%)	39 (33.3%)	23 (30.7%)
100-399	85 (18.9%)	29 (2.9%)	59 (20.1%)	29 (11.5%)	29 (24.8%)	6 (8.0%)
≥400	20 (4.5%)	1 (0.1%)	20 (6.8%)	1 (0.4%)	8 (6.8%)	1 (1.3%)
Total	*449 (100%)*	*1006 (100%)*	*294 (100%)*	*253 (100%)*	*117 (100%)*	*75 (100%)*

* Herds with <10 cattle were excluded from the study

Data collection

Data were gathered from face-to-face questionnaires and from surveys of badger setts and farm boundaries, with answers referring to the 12 months prior to the bTB breakdown for case farms and the 12 months prior to the survey taking place for control farms. Staff involved in data collection were trained in completing the questionnaires, conducting badger sett surveys, reading farm maps, use of camera/global positioning system (GPS) and recording of field boundaries. The majority of farms were visited by two members of staff to ensure consistency.

Two surveys of badger setts were conducted per farm; one survey evaluated the existence of badger activity within the farm boundary and a similar survey assessed the area within a 250 m radius around the farm buildings. Badger setts were classified according to Thornton (1998) (see Table 2).

Table 2: Badger sett classification according to Thornton (1998)

Sett Type	Characteristics
Main Sett	Usually a large number of entrances (both used and disused), large spoil heaps, well-worn paths and in continual use. There is one main sett per social group.
Annexe Sett	Close to main sett (<150m), several entrances that are generally less well used than main setts, conspicuous well-worn paths to the main sett, not necessarily in continual use.
Subsidiary Sett	Variable number of entrances although generally less than 4, usually not connected to other setts by well worn paths, partially used entrances, not continually used.
Outlier Sett	Generally just a single sett entrance (maximum 2) and not normally associated with well worn paths. Used sporadically.

Farm boundaries were defined as any place of contact with a contiguous neighbour; they were described by the participating farmers and boundary surveys were conducted for verification purposes. This resulted in an assessment of the possibility of nose-to-nose contact with cattle from the neighbouring farm, taking into account the farmer’s statement on whether cattle were grazed on both sides of the boundary over the 12 month period either prior to the bTB breakdown (cases) or over the 12 months prior to the survey taking place (controls) (risk assessment score 1 to 5; Table 3). If more than one boundary type was present between the study farm and a contiguous farm, they were recorded separately. In November 2010, a pilot study was conducted on eight farms, resulting in minor alterations to the questionnaire. The field study commenced in December 2010 and finished in June 2011.

Table 3: Distribution of the risk assessment scores for the farmer's and surveyor's assessment of the risk of nose-to-nose contact in the 12 months prior to the bovine tuberculosis (bTB) breakdown (cases) or in the 12 months prior to the survey taking place (controls) in a case-control study of 117 farms with a bovine tuberculosis breakdown and 75 farms without a breakdown in Northern Ireland in 2008/2009

			Farmer's risk assessment			Surveyor's risk assessment		
Risk score [a]	Nose-to-nose contact [a]	Description of farm boundary in relation to nose-to nose contact [a]	Case (n = 999)	Control (n = 584)	P value [b]	Case (n = 711)	Control (n = 454)	P value [b]
1	No direct nose-to-nose contact possible (min. gap between cattle ≥ 3 m)	Road or river of sufficient size to prevent nose-to-nose contact. Double fenced boundary with ≥ 3 m gap with or without an intervening hedge or other barrier.	527 (52.8%)	352 (60.3%)	<0.001	423 (59.5%)	293 (64.5%)	0.167
2	No direct nose-to-nose contact possible (min. gap between cattle < 3 m)	Double fenced boundary with ≥ 2 m gap with or without an intervening hedge or other barrier Thorn or other hedge with no obvious gaps, minimum width ≥ 2 m Laneway used by cattle, no obvious gaps	46 (4.6%)	23 (3.9%)		32 (4.5%)	25 (5.5%)	
3	Rare nose-to-nose contact possible	Laneway used by cattle, some gaps allowing direct nose to-nose contact Thorn or other hedge with no obvious gaps, minimum width < 2 m	217 (21.7%)	132 (22.6%)		130 (18.3%)	74 (16.3%)	

Table 3: continued

			Farmer's risk assessment			Surveyor's risk assessment		
Risk score [a]	Nose-to-nose contact [a]	Description of farm boundary in relation to nose-to nose contact [a]	Case (n = 999)	Control (n = 584)	P value [b]	Case (n = 711)	Control (n = 454)	P value [b]
4	Occasional nose-to-nose contact possible	Thorn or other hedge with occasional gaps allowing direct nose-to-nose contact (< 1% of hedge length) Thorn or other hedge with some gaps allowing direct nose-to-nose contact (1-10% of hedge length)	143 (14.3%)	65 (11.1%)		85 (12.0%)	47 (10.4%)	
5	Frequent nose-to-nose contact possible	Thorn or other hedge with frequent gaps allowing direct nose-to-nose contact (10-49% of hedge length) Fence only, with no or minimal hedging (hedge < 50% of length)	66 (6.6%)	12 (2.1%)		41 (5.8%)	15 (3.3%)	

[a] Farm boundaries were defined as any place of contact with a contiguous neighbour. The risk score of the farm boundary was described/assessed taking into account whether cattle had been grazed on both sides of the boundary over the 12 month period either prior to the bTB breakdown (cases) or the last 12 months prior to the survey taking place (controls). If more than one boundary type was present between the study farm and a contiguous farm they were separately recorded.

[b] P value based on likelihood ratio

Data handling and statistical analyses

Data were checked and entered on a SQL Server database. Data analyses were conducted using R (2.15.0; The R foundation for Statistical Computing) and SPSS Statistics version 19 (IBM). A comparison between farms that participated and the rest of the study population was conducted by location and herd size in order to assess response bias. There was no significant difference between participating case and control farms in relation to DVO area ($\chi^2 = 5.5703$; $P = 0.061$), but there was a significant difference in relation to herd size (two-sample *t* test; $P = 0.012$). Therefore, a univariable logistic regression model, with herd size added as an a priori confounder, was used to determine whether each variable was significantly associated with case-control status (dependent variable) for progression to multivariable analyses. Multivariable logistic regression was conducted, with variables with P values < 0.20 in the univariable analysis (based on the likelihood ratio test) being considered for inclusion (Mickey and Greenland, 1988; Katz, 2011). The decision whether to treat independent variables as continuous or categorical was determined by plotting each variable against the dependent variable. Collinearity between variables was assessed using correlation matrices (cut-off point 0.8; Katz, 2011). Final model selection was based on assessment of Akaike Information Criteria (AIC), model fit was assessed using the Hosmer-Lemeshow goodness-of-fit test ($P \leq 0.05$ indicating significant lack of fit to the data) and sensitivity analyses were conducted. Variables with low numbers in any of the categories (and where it was not possible to combine categories) were excluded (Katz, 2011). Biologically plausible two-way interactions between variables were added to the model and tested for significance ($P < 0.05$). A forward stepwise approach was used to build the final multivariable model. This approach is preferred for relatively small data sets containing large numbers of variables (Dohoo et al., 2009; Katz, 2011).

Results

An a priori sample size calculation (power = 0.8, ratio cases:controls = 1, precision = 0.95, predicted exposure in controls = 0.2, odds ratio, OR, detected = 2) resulted in a preferred sample size of 175 cases and 175 controls. Invited farmers (n = 547 (294 cases, 253 controls) received two follow-up telephone calls and one follow-up letter to encourage participation. In total, 192 farmers agreed to enrol, thus with a participation rate of 37.4% (n = 117) for cases and 32.4% (n = 75) for controls (see Fig. 2). Permission to conduct a farm boundary survey was obtained on 183 farms (95.3% of the total; 109 cases, 74 controls). Participating farms are described in Table 4.

Figure 2: Flow chart showing herd participation (case-control study of 117 and 75 farms with and without a bovine tuberculosis breakdown in Northern Ireland in 2008/2009)

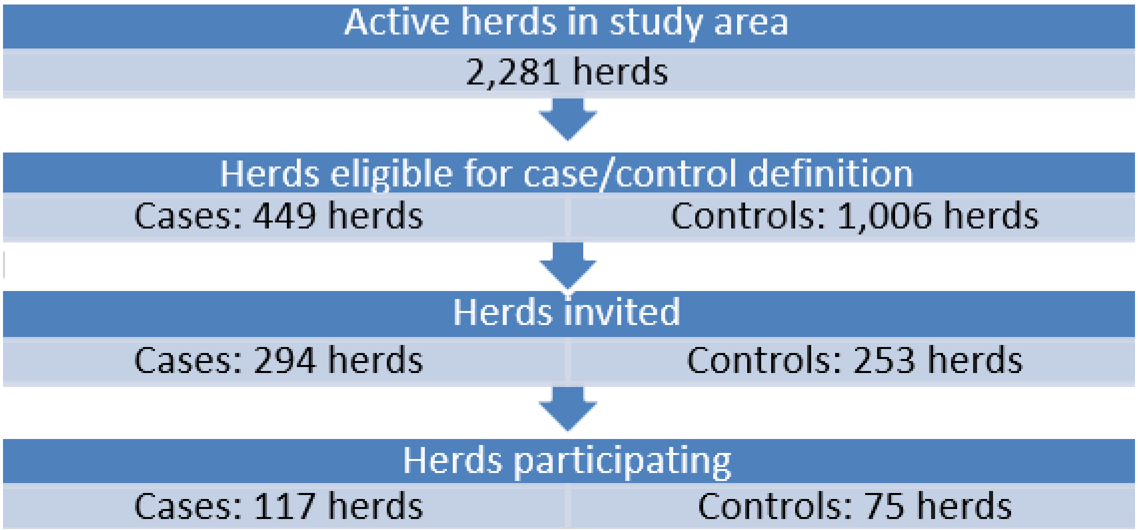


Table 4: Distribution of participating farms by herd size category, location and herd type (case-control study of 117 and 75 farms with and without a bovine tuberculosis breakdown in Northern Ireland in 2008/2009)

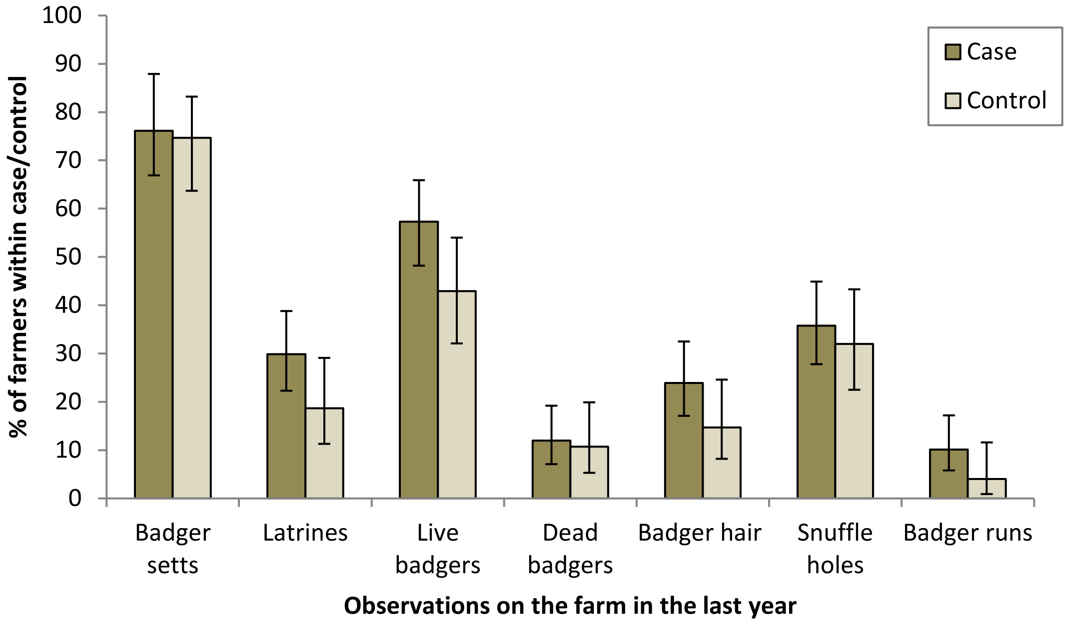
		Number cases (%) participating	Number controls (%) participating
Herd size category*	**10-19**	4 (3.4%)	2 (2.7%)
	20-29	4 (3.4%)	6 (8.0%)
	30-49	10 (8.5%)	8 (10.7%)
	50-99	23 (19.7%)	29 (38.7%)
	100-199	39 (33.3%)	23 (30.7%)
	100-399	29 (24.8%)	6 (8.0%)
	≥400	8 (6.8%)	1 (1.3%)
Location	**DVO 27**	38 (32.5%)	24 (32.0%)
	DVO 37	55 (47.0%)	25 (33.3%)
	DVO 39	24 (20.5%)	26 (34.7%)
Herd Type	**Beef**	77 (65.8%)	59 (78.7%)
	Dairy	20 (17.1%)	7 (9.3%)
	Mixed	20 (17.1%)	9 (12.0%)

* Herds with <10 cattle were excluded from the study

Key descriptives

Overall, 6.0% (95% confidence interval, CI, 2.7, 12.0) of case farmers and 9.3% (95% CI 4.3, 18.3) of control farmers actively looked for badger activity (see Fig. 3).

Figure 3: Observations of badgers and badger signs on the farm in the 12 months prior to the bTB breakdown (cases) or in the 12 months prior to the survey taking place (controls) as stated by the farmer with 95% confidence interval bars

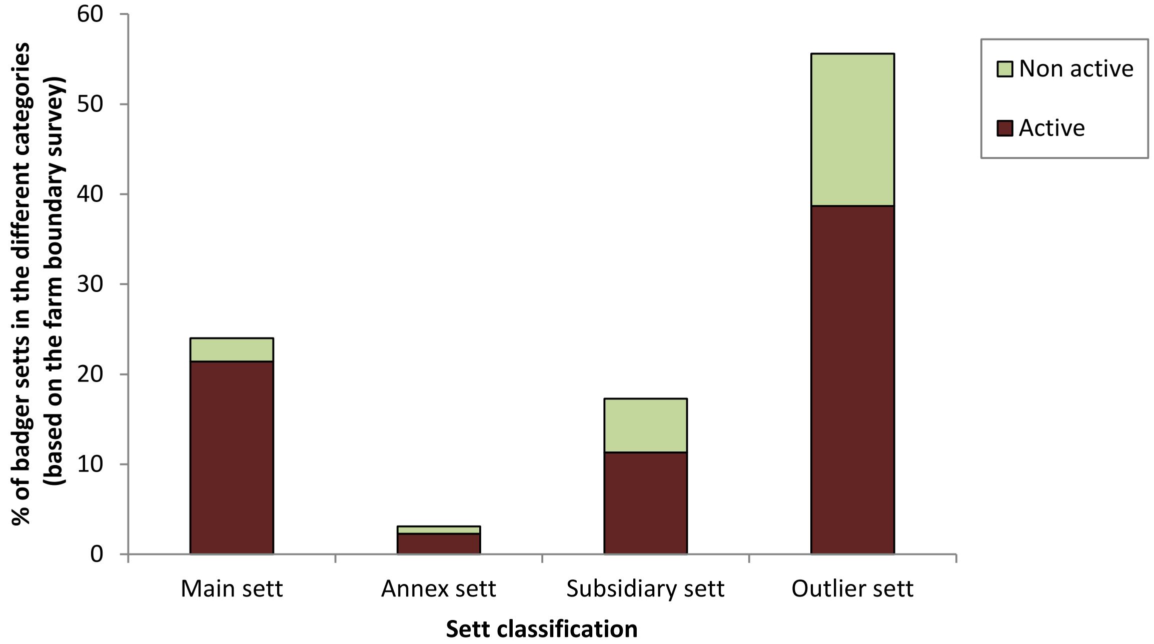


Surveyors found that the majority of farmer-reported badger setts were true badger setts (positive predictive value 81.4%; 95% CI 74.9, 86.5). Although 34.9% of farmers reported no badger setts on their farm, this was the case on only 17.2% of farms (negative predictive value 49.3%; 95% CI 37.7, 60.9). Of the setts within the farm boundary, most were stated to be accessible by cattle on case farms (82.0%; 95% CI 75.7, 86.9) and control farms (69.4%; 95% CI 58.9, 78.2; $\chi^2 = 5.3293$, $P = 0.021$). In total, 96.6% (95% CI 92.2, 98.8) of setts on case farms and 91.5% (95% CI 81.3, 96.7) of setts on control farms were in fields grazed by cattle in the year prior to the bTB breakdown (cases) or in the 12 months prior to the survey (controls) (Table 5; Fig. 4).

Table 5: Mean number and range of setts of different sett classifications found within the farm boundary and within a 250 m radius from farm buildings in a case-control study of 117 farms with a bovine tuberculosis breakdown and 75 farms without a breakdown in Northern Ireland in 2008/2009

	Case		Control		*P*
	Mean	Range	Mean	Range	(Two sample *t* test)
Setts found within farm boundary	1.56	0-7	1.13	0-4	0.301
Active setts found within farm	1.16	0-7	0.81	0-4	0.313
Main setts found within farm	0.41	0-3	0.21	0-2	0.151
Sett density (setts/ha farm land)	0.03	0-0.39	0.03	0-0.20	1.000
Active sett density (active setts/ha	0.01	0-0.39	0.02	0-0.08	1.000
Main sett density (main setts/ha	0.02	0-0.27	0.01	0-0.15	1.000

Figure 4: Distribution of badger sett classification in the study area



The key farm management and biosecurity descriptives are shown in Table 6.

Table 6: Key descriptives in relation to farm management and biosecurity measures in the 12 months prior to the bovine tuberculosis (bTB) breakdown (cases) or in the 12 months prior to the survey taking place (controls) in a case-control study of 117 farms with a bTB breakdown and 75 farms without a breakdown in Northern Ireland in 2008/2009

		Case (*n* = 117) % in category (95% CI)	Control (*n* = 75) % in category (95% CI)	*P* value [a]
Farm management				
Farm type	Beef farm	65.8% (56.8, 73.8)	78.7% (68.0, 86.5)	0.920
	Dairy farm	17.1% (11.3, 25.0)	9.3% (4.3, 18.3)	
	Mixed farm	17.1% (11.3, 25.0)	12.0% (6.2, 21.5)	
Let out land as conacre[b]		10.3% (5.8, 17.2)	13.3% (7.2, 23.0)	0.661
Take land as conacre[b]		83.8% (75.9, 89.4)	78.7% (68.0, 86.5)	0.821
Purchase cattle in the last 3 years		87.2% (79.8, 92.2)	90.7% (81.7, 95.7)	0.821
House cattle during the winter period only		94.0% (88.0, 97.3)	94.7% (86.7, 98.3)	0.375
Straw bedded accommodation for indoor housing available		79.5% (71.2, 85.9)	78.7% (68.0, 86.5)	0.566
Slatted accommodation for indoor housing available		76.1% (67.5, 82.9)	74.7% (63.7, 83.2)	0.313
Central feeding passage or feeding passage with enclosed sides		79.5% (71.2, 85.9)	73.3% (62.3, 82.1)	0.909
Rotational/paddock grazing		78.6% (70.3, 85.1)	78.7% (68.0, 86.5)	0.488
Feed big bales grass silage		75.2% (66.6, 82.2)	84.0% (73.9, 90.8)	0.381
Feed grass silage clamp		68.4% (59.5, 76.1)	62.7% (51.3, 72.8)	0.337
Feed meal at housing		94.9% (89.0, 97.9)	93.3% (85.0, 97.5)	0.859
Feed meal at grass		82.9% (76.1, 89.7)	76.0% (66.3, 85.7)	0.458
Feed meal in troughs at housing		76.9% (68.5, 83.7)	77.3% (66.6, 85.4)	0.455
Feed meal in troughs at grass		68.4% (59.5, 76.1)	65.3% (54.0, 75.1)	0.894
Observation of wild deer on the farm		29.1% (21.6, 37.9)	22.7% (14.6, 37.2)	0.290

Table 6: continued

	Case (*n* = 117) % in category (95% CI)	Control (*n* = 75) % in category (95% CI)	*P* value [a]
Biosecurity measures – badger related			
Use of electric fence to prevent badger access to clamps	0% (0, 3.8)	0% (0, 5.8)	-
Fence off badger latrines	5.1% (2.1, 11.0)	0% (0, 5.8)	-
Install solid gates/fencing around farmyard [c]	7.7% (3.9, 14.2)	8.0% (3.4, 16.7)	0.988
Fence off badger setts	11.9% (7.1, 19.2)	4.0% (0.9, 11.6)	0.091
Have solid doors which would prevent badger access to housing	38.5% (30.1, 47.5)	26.7% (17.9, 37.7)	0.083
Use raised water troughs at pasture [c]	61.5% (52.5, 69.9)	60.0% (46.7, 70.4)	0.906
Use raised feed troughs at pasture [c]	65.0% (56.0, 73.0)	64.0% (52.7, 74.0)	0.822
Badgers can access farmyard at night	95.7% (90.1, 98.4)	96.0% (88.4, 99.1)	0.998
Badgers can access feeding passage at night	72.2% (63.9, 79.9)	89.3% (80.1, 94.7)	0.035
Farm management related			
Arrangements with neighbours to avoid grazing contiguous fields	21.4% (14.9, 29.7)	17.3% (10.3, 27.6)	0.523
Maintain closed herd (i.e. no cattle purchases)	23.9% (17.1, 32.5)	24.0% (15.7, 34.9)	0.427
Avoid grazing boundary fields	52.1% (43.2, 61.0)	52.0% (40.9, 62.9)	0.740
Visitors have to wash/disinfect before entry	56.4% (47.4, 65.1)	48.0% (37.1, 59.1)	0.219
Routinely washing/disinfecting of cattle houses after emptying	86.3% (78.8, 91.5)	82.7% (72.4, 89.7)	0.436
Separate collection area for fallen livestock available [c]	88.0% (80.8, 92.9)	84.0% (73.9, 90.8)	0.633
No sharing of livestock equipment with other farmers [c]	88.0% (80.8, 92.9)	92.0% (83.3, 96.6)	0.191
Isolation accommodation for sick animals available	97.4% (92.4, 99.5)	98.6% (92.1, 100)	0.637

Table 6: continued

	Case (*n* = 117) % in category (95% CI)	Control (*n* = 75) % in category (95% CI)	*P* value [a]
Biosecurity measures			
Measures related to only herds that purchase cattle [d]	*n* = 89	*n* = 57	
Carry out post-movement test for bTB	2.2% (0.1, 8.3)	1.8% (0.0, 10.2)	0.751
Ask for pre-movement test for bTB	11.2% (6.0, 19.6)	8.8% (3.4, 19.4)	0.770
Isolation of purchased cattle for at least 21 days	40.4% (30.9, 50.8)	42.1% (30.2, 55.0)	0.984
Check bTB status prior to purchase	51.7% (41.5, 61.8)	49.1% (36.6, 61.7)	0.807

[a] *P* value based on likelihood ratio adjusted for herd size

[b] Conacre is the letting/taking of portions of land by or from a tenant

[c] These measures will improve biosecurity on the farm but may not necessarily be put in place by the farmer for that particular reason

[d] 89 case farms and 57 control farms purchased cattle stated not to be ‘closed’ herds (i.e. they purchased cattle)

Most farmers bought cattle in the last three years; case farmers bought a mean of 64 cattle and control farmers bought a mean of 44 cattle. There were means of 8.9 (range 2-33) boundaries per case farm and 8.2 (range 1-26) boundaries per control farm. On the basis of the surveyor's risk assessment, 66.8% of farm boundaries (case farms: 66.5%; 95% CI 62.7, 70.1; control farms: 67.4%; 95% 62.4, 72.1) provided opportunities for nose-to-nose contact (allowance of a minimum gap between cattle < 3 m) if cattle were grazed in fields on both sides of the farm boundary, which was in almost perfect agreement with the farmers' assessment (κ statistic = 0.788) (Landis and Koch, 1977). However, for 59.9% of boundaries as stated by the farmer (case farms: 57.3%; 95% CI 54.2, 60.4; control farms: 64.2%, 95% CI 60.3, 68.1) no direct nose-to-nose contact with neighbouring cattle was possible, taking into account whether cattle were grazed at the same time on both sides of the boundary in the last year (Table 7). This finding was also in near perfect agreement with the surveyors' assessments (κ statistic = 0.833).

Table 7: Distribution of the risk assessment scores for the farmer's and surveyor's assessment of the risk of nose-to-nose contact in the 12 months prior to the bovine tuberculosis (bTB) breakdown (cases) or in the 12 months prior to the survey taking place (controls) in a case-control study of 117 farms with a bovine tuberculosis breakdown and 75 farms without a breakdown in Northern Ireland in 2008/2009

			Farmer's risk assessment			Surveyor's risk assessment		
Risk score [a]	Nose-to-nose contact [a]	Description of farm boundary in relation to nose-to nose contact [a]	Case (n = 999)	Control (n = 584)	P value [b]	Case (n = 711)	Control (n = 454)	P value [b]
1	No direct nose-to-nose contact possible (minimum gap between cattle ≥ 3 m)	Road or river of sufficient size to prevent nose-to-nose contact. Double fenced boundary with ≥ 3 m gap with or without an intervening hedge or other barrier.	527 (52.8%)	352 (60.3%)	<0.001	423 (59.5%)	293 (64.5%)	0.167
2	No direct nose-to-nose contact possible (minimum gap between cattle < 3 m)	Double fenced boundary with ≥ 2 m gap with or without an intervening hedge or other barrier Thorn or other hedge with no obvious gaps, minimum width ≥ 2 m Laneway used by cattle, no obvious gaps	46 (4.6%)	23 (3.9%)		32 (4.5%)	25 (5.5%)	
3	Rare nose-to-nose contact possible	Laneway used by cattle, some gaps allowing direct nose to-nose contact Thorn or other hedge with no obvious gaps, minimum width < 2 m	217 (21.7%)	132 (22.6%)		130 (18.3%)	74 (16.3%)	
4	Occasional nose-to-nose contact possible	Thorn or other hedge with occasional gaps allowing direct nose-to-nose contact (< 1% of hedge length) Thorn or other hedge with some gaps allowing direct nose-to-nose contact (1-10% of hedge length)	143 (14.3%)	65 (11.1%)		85 (12.0%)	47 (10.4%)	

Table 7: continued

Risk score [a]	Nose-to-nose contact [a]	Description of farm boundary in relation to nose-to nose contact [a]	Farmer's risk assessment Case (*n* = 999)	Control (*n* = 584)	P value[b]	Surveyor's risk assessment Case (*n* = 711)	Control (*n* = 454)	P value[b]
5	Frequent nose-to-nose contact possible	Thorn or other hedge with frequent gaps allowing direct nose-to-nose contact (10-49% of hedge length) Fence only, with no or minimal hedging (hedge < 50% of length)	66 (6.6%)	12 (2.1%)		41 (5.8%)	15 (3.3%)	

[a] Farm boundaries were defined as any place of contact with a contiguous neighbour. The risk score of the farm boundary was described/assessed taking into account whether cattle had been grazed on both sides of the boundary over the 12 month period either prior to the bTB breakdown (cases) or the last 12 months prior to the survey taking place (controls). If more than one boundary type was present between the study farm and a contiguous farm they were separately recorded.

[b] *P* value based on likelihood ratio.

Multivariable analyses

The final multivariable logistic regression model is displayed in Table 8. The model fitted the data well (Hosmer-Lemeshow test: $P > 0.05$) and the sensitivity was 76.1%. The statistical significance of farms that used contractors to spread manure or slurry on their farm having higher odds of being a case was particularly based on spreading slurry (41.0% of cases vs. 21.3% of controls) rather than manure (23.9% of cases vs. 17.3% of controls). Few farmers (27.1% of cases; 31.8% of controls) stated that contractors that they used washed their equipment before arrival on the farm.

Table 8: Multivariable logistic regression model for risk factors for occurrence of recent bovine tuberculosis (bTB) breakdown in cattle herds in case-control study of 117 farms with a bTB breakdown and 75 farms without a breakdown in Northern Ireland in 2008/2009.

Variable	Cases (*n* = 117) *n*	%	Controls (*n* = 75) *n*	%	Odds ratio	95% Confidence interval	*P* value Wald test	*P* value Likelihood ratio
Herd size [a]								0.003
Per animal increase					1.01	1.00, 1.01	0.006	
Accessible badger sett within farm boundaries and in a field grazed in the last 12 months [b]								
Not present	48	41.0%	48	64.0%	1.00	-	-	< 0.001
Present	69	59.0%	27	36.0%	4.14	1.79, 9.55	< 0.001	
Live badgers								
Not observed in the last 3 years [c]	54	46.2%	46	61.3%	1.00	-	-	0.019
Observed in the last 3 years [c]	63	53.8%	29	38.7%	2.51	1.15, 5.48	0.021	
Feeding troughs in housing								
Not accessible to badgers	74	63.2%	41	54.7%	1.00	-	-	0.005
Accessible to badgers	43	36.8%	34	45.3%	0.31	0.14, 0.73	0.007	
Does farmer feel badgers could access the house/feeding passage at night								
No	29	24.8%	8	10.7%	1.00	-	-	0.022
Yes	88	75.2%	67	89.3%	0.30	0.11, 0.88	0.028	
Purchase of store/beef cattle in last 3 years [c]								
No	89	76.1%	62	82.7%	1.00	-	-	0.003
Yes	28	23.9%	13	17.3%	4.60	1.61, 13.13	0.004	
Use of contractors to spread slurry or manure on farm								
No	58	49.6%	52	69.3%	1.00	-	-	0.011
Yes	59	50.4%	23	30.7%	2.83	1.24, 6.49	0.014	
Storage of processed grain or compound meal on the farm								
No	16	13.7%	5	6.7%	1.00	-	-	0.002
Yes	101	86.3%	70	93.3%	0.12	0.03, 0.52	0.004	

Table 8: Continued

Variable	Cases (*n* = 117) *n*	%	Controls (*n* = 75) *n*	%	Odds ratio	95% Confidence interval	*P* value Wald test	*P* value Likelihood ratio
Meal fed on top of silage in housing								
No	66	56.4%	52	69.3%	1.00	-	-	0.002
Yes	51	43.6%	23	30.7%	3.55	1.53, 8.23	0.003	
Magnesium fed as a supplement								
No	84	71.8%	63	84.0%	1.00	-	-	0.006
Yes	33	28.2%	12	16.0%	3.77	1.39, 10.17	0.009	
Lambs on the farm								
Not present	97	82.9%	70	93.3%	1.00	-		0.004
Present	20	17.1%	5	6.7%	5.47	1.58, 19.02	0.007	
Poultry on the farm								
Not present	113	96.6%	61	81.3%	1.00	-	-	< 0.001
Present	4	3.4%	14	18.7%	0.14	0.04, 0.49	0.002	

[a] Included in the model as a continuous variable.

[b] In the last 12 months refers to the 12 months prior to the bTB breakdown for cases and to the 12 months prior to the survey taking place for controls.

[c] In the last 3 years refers to the 3 years prior to the bTB breakdown for cases and to the 3 years prior to the survey taking place for controls.

Discussion

Although the knowledge of risk factors in relation to bTB breakdown is vast (Skuce et al., 2012), there is, as yet, no empirical evidence linking improved biosecurity with reduced risk of bTB breakdown. The current study therefore focussed on risks that could be mitigated by improved bTB related biosecurity and furthermore assessed the uptake of such measures by farmers in Northern Ireland. It was not possible to assess bTB history as a risk factor within this study, as identified in previous studies (Olea-Popelka et al, 2004; Porphyre et al., 2008; Abernethy et al., 2010), since bTB history was used to select cases and controls.

The purchase of beef cattle was highlighted as a risk factor in the final model. Since only 24% of farmers stated that they had a closed farm and, because pre-movement testing is not compulsory and seldom conducted, the purchase of cattle is a plausible potential source of bTB introduction into a herd, consistent with previous studies (Barlow et al., 1998; Goodchild and Clifton-Hadley, 2001; Gilbert et al., 2005; Gopal et al., 2006; Clegg et al., 2008; Green et al., 2008).

The use of contractors to spread slurry or manure was also associated with being a case farm, consistent with findings by Griffin et al. (1993) and Wolfe et al. (2010). This is plausible, since *M. bovis* can survive in faeces for several months, aerosol can be created by slurry and few farmers stating that contractors wash and disinfect their equipment after use.

Contact with neighbouring cattle (Phillips et al., 2003) was possible for 66.8% of farm boundaries in our study and 79% in a previous study (Denny and Wilesmith, 1999). However, we found no significant association between being a case farm and the number of boundaries per farm, as well as mean risk scores based on nose-to-nose contact and farm boundary nature; this is in contrast to findings reported by Denny and Wilesmith (1999), Munroe et al. (1999), Karolemeas et al. (2010) and Johnston et al. (2011). Since the current study examined the overall effect of contact with neighbouring herds, instead of focussing on neighbouring herds with bTB breakdowns, there may have been a dilution of the possible association between the risk of bTB breakdown and contact with contiguous herds.

No significant association was found between the presence of deer and the risk of being a case farm, consistent with previous findings by Denny and Wilesmith (1999). The prevalence of *M. bovis* in wild deer in Northern Ireland is known to be low

(~2%[1]). Farmers that kept poultry or lambs as well as cattle were at a lower and higher risk, respectively, of being a case farm, for which the explanation is unclear. The positive and negative predictive values of farmers identifying badger setts correctly were 81.4% and 49.3%, respectively, compared to the values of 95% and 30% reported by Menzies et al. (2011). The odds of being a case farm were significantly increased by the presence of accessible badger setts and the observation of live badgers on the farm. Badgers are attracted by food and feed supplements, and have been reported to visit feed stores and cattle houses (Phillips et al., 2003; Tolhurst et al., 2009; Ward et al., 2010; O'Mahoney, 2014)[2]. Significantly fewer case farmers stored processed grain on their farm in comparison with control farmers, but there was no difference in how they stored this grain and how accessible it was to badgers. Case farmers were significantly more likely to feed magnesium supplement and meal on top of the silage, which may attract badgers. However, case farmers were more confident that their cattle houses and feeding troughs were badger proof, although they did not report putting more badger proofing measures in place in comparison with control farmers.

In line with findings by Ward et al. (2010), there was a lack of adoption of biosecurity measures by farmers, especially in relation to badgers. Although most study farms used raised feed and water troughs, they were likely to have been installed for reasons other than increasing biosecurity (e.g. to prevent cattle defecating into troughs). Little was done (pre- or post-movement testing, maintaining a closed herd) on both case and control farms to reduce the risk of bTB introduction by cattle purchase. Isolation, washing and disinfection were better adopted.

Since the current study had a lower than anticipated level of farmer participation, there may be a possibility of participation bias and reduced study power. However, the participation rates of invited case and control farmers were similar. Participating farms were compared to the study population and a significant difference in mean herd size between cases and controls was evident; this was accounted for by adding herd size to all derived models as an a priori confounder.

[1] See: https://www.dardni.gov.uk/articles/wild-deer-tb-surveillance-200809 (accessed 22 March 2016).
[2] See: https://www.dardni.gov.uk/publications/badger-cattle-interactions-rural-environment-implications-bovine-tuberculosis (accessed 22 March 2016).

The risk of response bias was mitigated by the use of surveying staff, who verified farmers' responses (presence of badger setts and nature of farm boundaries), which showed a high level of agreement. Furthermore, several survey questions overlapped, allowing additional validation. The majority of farms were visited by two members of staff, which further ensured consistency. Some response bias was suspected on the basis of some of the answers to the questionnaire by case farmers, perhaps related to a reluctance to attribute responsibility for bTB breakdowns on their farms.

Since the questionnaires were based on answers referring to the 12 months prior to the bTB breakdown for case farms, but the last 12 months for control farms, the time period for recall for case farmers was longer than for control farmers, possibly leading to recall bias. However since bTB breakdowns for the case farms were recent (2008/2009) compared to the survey being conducted (2010/2011), this is not likely to have a major impact. Furthermore, some minor changes in the classification of some of the setts may have occurred between time badger setts were surveyed (2010-2011) and the time of the bTB breakdowns (2008-2009) on case farms.

Conclusions

The risk of several significant factors associated with being a case farm potentially can be reduced by adopting suitable biosecurity measures, particularly in relation to cattle purchases (e.g. keeping a closed herd and pre-movement testing) and badger proofing of the farm. The results of the current study should be applicable to other locations with farming practices that are similar to Northern Ireland, such as other areas of the British Isles.

Conflict of interest statement

None of the authors has any financial or personal relationships that could inappropriately influence or bias the content of the paper.

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References

Abernethy, D.A., Denny, G.O., Menzies, F.D., McGuckian, P., Honhold, N., Roberts, A.R., 2006. The Northern Ireland programme for the control and eradication of *Mycobacterium bovis.* Veterinary Microbiology 112, 231-237.

Abernethy, D.A., Graham, D., Skuce, R., Gordon, A., Menzies, F., Robinson, P., Harwood, R., Clarke, N., 2010. Shifting the TB-control paradigm: An epidemiological critique of the bovine tuberculosis eradication scheme. Proceedings of the Society of Veterinary Epidemiology and Preventive Medicine, Nantes, France, pp. 166-173.

Barlow, N.D., Kean, J.M., Caldwell, N.P. Ryan, T.J., 1998. Modelling the regional dynamics and management of bovine tuberculosis in New Zealand cattle herds. Preventive Veterinary Medicine 31, 57-75.

Brennan, M.L., Christley, R., 2012. Biosecurity on cattle farms: A study in North-West England. PLoS One 7, e28139.

Brandt, A.W., Sanderson, M.W., DeGroot, B.W., Thomson, D.U., Hollis, L.C., 2008. Biocontainment, biosecurity, and biosecurity practices in beef feedyards. Journal of the American Veterinary Medical Association 232, 262-269.

Clegg, T.A., More, S.J. Higgins, I.M., Good, M., Blake, M. Williams, D.H., 2008. Potential infection-control benefit for Ireland from pre-movement testing of cattle for tuberculosis. Preventive Veterinary Medicine 84, 94-111.

Clegg, T.A., Duignan, A., Whelan, C., Gormley, E., Good, M., Clarke, J., Toft, N. More, S.J. 2011. Using latent class analysis to estimate the test characteristics of the γ-interferon test, the single intradermal comparative tuberculin test and a multiplex immunoassay under Irish condition. Veterinary Microbiology 151, 68-76.

Cresswell, E., Brennan, M.L., Barkema, H.W., Wapenaar, W., 2014. A questionnaire based survey on the uptake and use of cattle vaccines in the UK. Veterinary Record Open 1, e000042.

Denny, G.O., Wilesmith, J.W., 1999. Bovine tuberculosis in Northern Ireland: A case-control study of herd risk factors. Veterinary Record 144, 305-310.

Dohoo, I., Martin, W. Stryhn, H., 2009. Veterinary Epidemiologic Research, 2nd Edn. VER, Charlottetown, Prince Edward Island, Canada, 865 pp.

Durr, P.A. Froggatt, A.E., 2002. How best to geo-reference farms? A case study from Cornwall, England. Preventive Veterinary Medicine 56, 51-62.

Gilbert, M., Mitchell, A., Bourn, D., Mawdsley, J., Clifton-Hadley, R. Wint, W. 2005. Cattle movements and bovine tuberculosis in Great Britain. Nature 435, 491-496.

Goodchild, A.V., Clifton-Hadley, R.S., 2001. Cattle-to-cattle transmission of *Mycobacterium bovis.* Tuberculosis (Edinb.) 81, 23-41.

Gopal, R., Goodchild, A, Hewinson, G., de la Rua-Domenech, R. Clifton-Hadley, R., 2006. Introduction of bovine tuberculosis to north-east England by bought-in cattle. Veterinary Record 159, 265-271.

Green, L.E., Cornell, S.J., 2005. Investigations of cattle herd breakdowns with bovine tuberculosis in four countries of England and Wales using VetNet data. Preventive Veterinary Medicine 70, 293-311.

Green, D.M., Kiss, I.Z., Mitchell, A.P., Kao, R.R., 2008. Estimates for local and movement based transmission of bovine tuberculosis in British cattle. Proceedings of the Royal Society B Biological Sciences 275, 1001-1005.

Griffin, J.M., Hahesy, T., Lynch, K., Salman, M.D., McCarthy, J. Hurley, T., 1993. The association of cattle husbandry practices, environmental factors and farmer characteristics with the occurrence of chronic bovine tuberculosis in dairy herds in the Republic of Ireland. Preventive Veterinary Medicine 17, 145-160.

Griffin, J.M., Martin, S.W., Thorburn, M.A., Eves, J.A., Hammond, R.F., 1996. A case-control study on the association of selected risk factors with the occurrence of bovine tuberculosis in the Republic of Ireland. Preventive Veterinary Medicine 27, 217-229.

Johnston, W.T., Vial, F., Gettinby, G., Bourne, F.J., Clifton-Hadley, R.S., Cox, D.R., Crea, P., Donnelly, C.A., McInerney, J.P., Mitchell, A.P. et al., 2011. Herd-level risk factors of bovine tuberculosis in England and Wales after the 2001 foot-and-mouth disease epidemic. International Journal of Infectious Diseases 15, e833-e840.

Judge, J., McDonald, R.A., Walker, N., Delahay, R.J., 2011. Effectiveness of biosecurity measures in preventing badger visits to farm buildings. PLoS ONE 6: e28941

Katz, M.H., 2011. Multivariable Analysis: A Practical Guide for Clinicians, 3rd Edn. Cambridge University Press, Cambridge, UK, 250 pp.

Karolemeas, K., McKinley T.J., Clifton-Hadley, R.S., Goodchild, A.V., Mitchell, A., Johnston, W.T., Conlan, A.J., Donnelly, C.A., Wood, J.L, 2010. Predicting prolonged bovine tuberculosis breakdowns in Great Britain as an aid to control. Preventive Veterinary Medicine 97, 183-190.

Landis, J.R., Koch, G.G., 1977. The measurement of observer agreement for categorical data. Biometrics 33, 159-174.

Menzies, F.D., Abernethy, D.A., Stringer, L.A., Jordan, C., 2011. A comparison of badger activity in two areas of high and low bovine tuberculosis incidence of Northern Ireland. Veterinary Microbiology 151, 112-119.

Mickey, R.M., Greenland, S., 1989. The impact of confounder selection criteria on effect estimation. American Journal of Epidemiology 129, 125-137.

Munroe, F.A., Dohoo, I.R., McNab, W.B. Spangler, L., 1999. Risk factors for the between-herd spread of *Mycobacterium bovis* in Canadian cattle and cervids between 1985 and 1994. Preventive Veterinary Medicine 41, 119-133.

Nöremark, M., Frossling, J., Lewerin, S.S., 2010. Application of routines that contribute to on-farm biosecurity as reported by Swedish livestock farmers. Transboundary and Emerging Diseases 57, 225-236.

O'Hagan, M.J.H., Courcier, E.A., Drewe, J.A., Gordon, A.W., McNair, J., Abernethy, D.A. 2015. Risk factors for visible lesions or positive laboratory tests in bovine tuberculosis reactor cattle in Northern Ireland. Preventive Veterinary Medicine 120, 283-290

O'Hagan, M.J.H., Matthews, D.I., Laird, C., McDowell, S.W.J., 2016. Farmers' beliefs about bovine tuberculosis control in Northern Ireland. The Veterinary Journal doi: 10.1016/j.tvjl.2015.10.038.

Olea-Popelka, F.J., White, P.W., Collins, J.D., O'Keeffe, J., Kelton, D.F., Martin, S.W., 2004. Breakdown severity during a bovine tuberculosis episode as a predictor of future herd breakdowns in Ireland. Preventive Veterinary Medicine 63, 163-172.

Phillips, C.J.C., Foster, C.R.W., Morris, P.A., Teverson, R., 2003. The transmission of *Mycobacterium bovis* infection to cattle. Research in Veterinary Science 74, 1-5.

Pollock, J.M., Neill, S.D, 2002. *Mycobacterium bovis* infection and tuberculosis in cattle. The Veterinary Journal 163, 115-127.

Porphyre, T. Stevenson, M.A., McKenzie, J., 2008. Risk factors for bovine tuberculosis in New Zealand cattle farms and their relationship with possum control strategies. Preventive Veterinary Medicine 86, 93-106.

Sayers, R.G., Sayers, G.P., Mee, J.F., Good, M., Bermingham, M.L., Grant, J., Dillon, P.G., 2013. Implementing biosecurity measures on dairy farms in Ireland. The Veterinary Journal 197, 259-267.

Skuce, R.A., Allen, A.R., McDowell, S.W.J., 2012. Herd-level risk factors for bovine tuberculosis: A literature review. Veterinary Medicine International 2012, 621210.

Thornton, P. S., 1998. Density and distribution of badgers in South West England - a predictive model. Mammal Review 18, 11-23.

Tolhurst, B.A., Delahay, R.J.., Walker, A.I., Ward, A.I., Roper, T.J., 2009. Behaviour of badgers (*Meles meles*) in farm buildings: Opportunities for the transmission of *Mycobacterium bovis* to cattle. Applied Animal Behaviour Science 117, 103-113.

Ward, A.I., Judge, J., Delahay, R.J., 2010. Farm husbandry and badger behaviour: Opportunities to manage badger to cattle transmission of *Mycobacterium bovis*? Preventive Veterinary Medicine 93, 2-10

Wilson, G.J., Carter, S. P., Delahay, R.J., 2011. Advances and prospects for management of TB transmission between badgers and cattle. Veterinary Microbiology 151, 43-50

Wolfe, D.M., Berke, O., Kelton, D.F., White, P.W., More, S.J., O'Keeffe, J., Martin, S.W., 2010. From explanation to prediction: A model for recurrent bovine tuberculosis in Irish cattle herds. Preventive Veterinary Medicine 94, 170-177

Chapter 5

The impact of the number of tuberculin skin test reactors and infection confirmation on the risk of future bovine tuberculosis incidents; a Northern Ireland perspective

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Abstract

Currently policies enabling cattle herds to regain Official Tuberculosis Free (OTF) status after a bovine tuberculosis (bTB) herd incident vary between individual parts of the British Isles from requiring only one negative Single Comparative Intradermal Tuberculin Test (SCITT) herd test when bTB infection is not confirmed to needing two consecutively negative SCITT herd tests after disclosure of two or more reactors, irrespective of bTB confirmation. This study used Kaplan Meier curves and univariable and multivariable Cox Proportional Hazard models to evaluate the effect of the number of SCITT reactors and bTB confirmation on the risk of future bTB herd incident utilising data extracted from the national animal health database in Northern Ireland. Based on multivariable analyses the risk of a future bTB herd incident was positively associated with the number of SCITT reactors identified during the incident period (Hazard Ratio=1.861 in incidents >5 SCITT reactors compared to incidents with only one SCITT reactor; P<0.001), but not with bTB confirmation. These findings suggest that the probability of residual bTB infection in a herd increases with an increasing number of SCITT reactors disclosed during a bTB herd incident. It was concluded that bTB herd incidents with multiple SCITT reactors should be subjected to stricter control measures irrespective of bTB infection confirmation status.

Keywords: Cattle; *Mycobacterium bovis*; Survival analyses; Risk factors; TB confirmation

Introduction

Bovine tuberculosis (bTB) is an infectious disease caused by *Mycobacterium bovis*, a zoonotic organism that affects cattle and many other mammals. Cattle are most likely to get infected through inhalation of aerosolised droplet nuclei [1, 2]. Once *M. bovis* has entered the bronchioles/alveoli, multiplication occurs and lesions are formed [3].

The single comparative intradermal tuberculin test (SCITT) is the main ante-mortem surveillance tool for bTB in European cattle. In Northern Ireland, all cattle over 6 weeks are tested annually with the SCITT and there is compulsory slaughter of cattle that are SCITT reactors [4]. EU legislation (European Directive 64/432/EEC (as amended)) requires post-mortem and bacteriological examination of SCITT reactors where bTB has not previously been confirmed during a bTB herd incident. In order to confirm bTB, samples from SCITT reactors identified with gross bTB-like visible lesions at post-mortem inspection are subjected to histological examination. If no histological evidence consistent with bTB are found, these samples are subjected to bacteriological culture, as are samples of bronchial and mediastinal lymph nodes from SCITT reactors with no bTB-like visible lesions [5].

Under natural circumstances, it can take several months for infected animals to develop lesions of bTB sufficiently large to be visible at post mortem examination. Due to this delay, the cellular immune response, which is measured by the SCITT, can be detected much earlier than gross pathology. Furthermore, the detection of lesions by visual examination at the slaughter house has been shown to be insensitive [6, 7]. In Northern Ireland, 43% of SCITT reactors animals were found to have visible lesions considering the years 1998, 2002 and 2006. The likelihood of *M. bovis* confirmation in an infected animal is greatly increased by sampling from visible lesions, with 99.8% of SCITT reactors with visible lesions being confirmed by histopathology or culture, whereas only 4.3% of non-visibly lesioned SCITT reactors are confirmed by these laboratory tests [5]. Failure to isolate *M. bovis* during post-mortem examination however does not necessarily mean that the animal has not been excreting the organism [1].

Animals infected with environmental mycobacteria can also react positively to the SCITT, but there will normally be no evidence of bTB related visible lesions. However, the specificity of the SCITT has been estimated at over 99.9%, indicating that a SCITT false positive result is a rare event [8, 9]. The sensitivity of the SCITT shows great variation; a median of 80% (range 75-96%) at standard interpretation, highlighting the risk of residual infection in herds has been reported [8]. Bayesian analytical techniques have suggested even lower SCITT sensitivity levels (50%) [10]. Currently policies relating to requirements for cattle herds to regain Official Tuberculosis Free status (OTF) after a bTB incident vary across the British Isles from only requiring one negative herd-level SCITT if infection is not confirmed (termed OTS regimen; as outlined in European Directive 64/432/EEC, as amended), to requiring two consecutively negative herd-level SCITTs (termed OTW regimen) after disclosure of two or more SCITT reactors, even if no confirmation of infection is found. In Northern Ireland, at the time of writing herds with 5 or less unconfirmed SCITT reactors need only one clear herd-level SCITT, whereas all other incidents require two clear consecutive herd-level SCITTs at intervals of 42–60 days in order to regain OTF status.

The number of SCITT reactors is strongly correlated to the confirmation status of the bTB herd incident [11]. A study conducted in the Republic of Ireland [12], showed that the risk of a future bTB incident episode was found to increase with incident severity (as measured by grouped numbers of standard SCITT reactors) and not with the presence of (confirmed) visible lesions. These findings were later confirmed in other studies [13-16]. The current study took a different approach as it focused on the specific number of SCITT reactors during the bTB herd incident taking confirmation status into account as a risk factor for future bTB herd incident. The aim of the study was to determine an appropriate cut-off point of number of SCITT reactors during a bTB herd incident, beyond which the OTW regimen should be applied in order for the herd to regain OTF status. The hypothesis tested was that the probability that one or more bTB infected animals will remain in the herd after a single, negative SCITT herd test increased with an increasing number of SCITT reactors during a bTB herd incident.

Materials and Methods

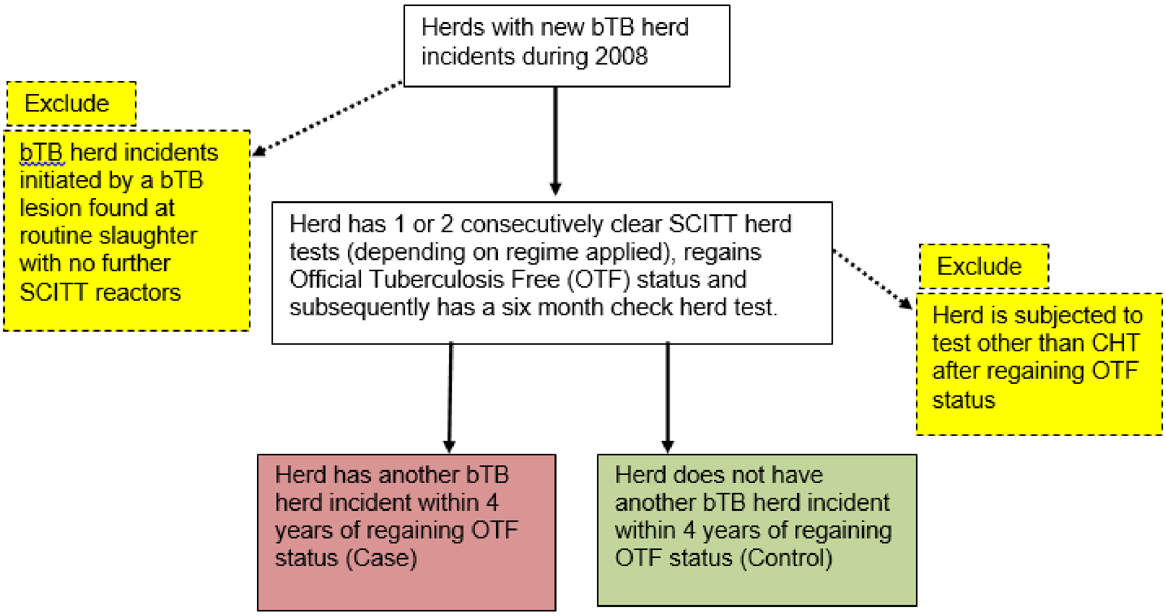
Study design and study population

An observational, retrospective cohort study was conducted with the study population including all new bTB herd incidents occurring during 2008 that had a six-month follow-up herd-level SCITT Check Herd Test (CHT) after withdrawal of movement restrictions following the bTB incident. Herds that had an initial herd-level SCITT follow-up that was not a CHT were excluded from the analysis as they may have been at increased risk from other factors such as being contiguous to another bTB herd incident. New bTB herd incidents that were initiated by bTB detection in an animal at routine slaughter without any subsequent SCITT reactors were also excluded. A new bTB herd incident for the sampling frame was defined as a herd that had one or more SCITT reactors in 2008 with no SCITT reactors during the previous 12 months. Study herds were followed up for a four year period after regaining OTF status following the end of the initial bTB herd incident (Figure 1). Within that four year follow-up period a herd was considered to be a bTB incident again if SCITT reactors or bTB detection in animals at routine slaughter were disclosed. The methodology used for the survival study was broadly based on a study design previously used in the Republic of Ireland [12].

Data collection and definition of variables

All data were extracted from the Animal and Public Health Information System of the Department of Agriculture, Environment and Rural Affairs. This database includes details on all individual cattle, their inter-herd movements and SCITT tests conducted since 1988 [17]. Datasets were merged and manipulated using MS Access™ 2007 and subsequently analysed using R 3.3.3. (The R Foundation for Statistical Computing; 'survival' R package [18]).

Figure 1: Diagram outlining the study design



Variables included in the analyses were based on characteristics of the initial bTB herd incident in 2008. They included the number of SCITT reactors (over the entire bTB herd incident period and at the disclosing SCITT), bTB confirmation, herd size, bTB history in the previous 3 years, Divisional Veterinary Office (DVO), herd type, local bTB prevalence and animal purchase intensity. These variables are defined below.

The number of SCITT reactors at the disclosing test and during the bTB herd incident was based on all animals defined as SCITT reactors with the baseline set as one SCITT reactor during the bTB herd incident. This is in contrast to other studies who compared bTB herd incidents with a baseline of herds that were clear of bTB [12] or who compared herds with different categories of number of SCITT reactors during the bTB herd incident with baseline herds that had bTB detected by visible lesions in animals at routine slaughter with no further SCITT reactors [15]. In the current study, the categories 1, 2, 3, 4, 5, >5 SCITT reactors were chosen in order to try and identify a justifiable cut-off point for having to implement OTW rather than OTS regimen in order to regain OTF status relating back to the current policy in the Northern Ireland bTB programme.

Confirmation of bTB infection was based on positive histology and/or bacteriological culture in samples from SCITT reactors after slaughter [5]. Herd size was based on the average number of animals tested at herd-level SCITTs in the 3 years prior to the initial disclosure SCITT. The bTB history of the herd was a binomial variable being positive if at least one SCITT reactor (confirmed or unconfirmed) or animal with a confirmed bTB lesion at routine slaughter had been identified in the 3 years prior to the initial SCITT disclosure. Herd type was also a binomial variable (dairy/non-dairy) based on the herd possessing a milk licence. Northern Ireland is divided in 10 administrative areas called Divisional Veterinary Office (DVO) areas, each of which are under the veterinary management of a divisional veterinary officer with smaller geographical areas or 'patches' that are managed by veterinary officers. The DVO area where the herd was located was included as an explanatory variable in order to adjust for regional differences. Local bTB prevalence was based on the herd prevalence in the patch area during the year that the CHT took place. The purchase intensity was based on the number of animals purchased in 90 days before either the start date of the next bTB herd incident during the follow-up period or the end date of the survival period in herds where there was no bTB herd incident during the follow-up period. This definition was similar to the definition for purchase intensity used in previous research [19].

Data analyses

Survival analyses based plots of the Kaplan-Meier estimators were conducted to evaluate the survival rate of study herds [20], focused on the number of SCITT reactors during the bTB herd incident and the bTB confirmation status of the incident. Further survival analyses were conducted on a subset of data based on bTB herd incidents that had no further SCITT reactors after the disclosure test (categorized by number of reactors).

In order to control for potential confounders, Cox regression univariable and multi-variable models were constructed [21]. Continuous explanatory variables were assessed whether they should be included in the analyses with or without categorization, by comparing their lowess (locally weighted scatter plot smoothing) curve with a linear regression line [22]. If there was no significant departure of the linear regression line from the lowess curve, the explanatory variable was entered to the model as being continuous. If the variable could not be entered as a continuous variable, it was categorised using biologically appropriate cut off points or quartiles, as appropriate.

Specifically, herd size was included as a continuous variable, purchase intensity was divided into 5 categories with no cattle purchases in the previous 90 days being a separate category, representing ‘closed herds’ and the remaining data being divided into quartiles. Patch bTB prevalence was divided into quartiles.

Univariable analyses were carried out on each explanatory variable and they were entered into the multivariable model if they were associated with the outcome at a p value of <0.200 using a forward step-wise method [22]. The best model was chosen based on Akaike Information Criterion (AIC) values [22, 23]. A correlation matrix was constructed of all pair wise combinations of variables in order to assess collinearity. All combinations of two-way interactions were assessed. The linearity in the log hazard function over time was assessed by categorizing the continuous variables into multiple dichotomous variables of equal units. These variables were entered into the analyses and each coefficient was graphed against the midpoint of the variable in order to assess linearity [22]. The proportional hazard assumption was tested using Schoenfeld residuals [24]. The power of the study encompassing 408 events (i.e. future bTB herd incidents) was deemed to be sufficient for multivariable analyses as at a maximum 30 covariates, only 300 events are required [23, 25]. A cut off point of $P<0.05$ was considered to be statistically significant in both univariable and multivariable models.

Results

Descriptive results

There were 1,036 new bTB herd incidents during 2008 that had a six-month follow-up herd-level SCITT Check Herd Test (CHT) after derestriction from the bTB herd incident. Of those herds, 408 (39.4%) had a future bTB herd incident within the follow-up time.

Descriptive results by number of SCITT reactors during the initial bTB herd incident are displayed in Table 1. The absolute risk increased whereas the median time to a future incident decreased by increasing number of SCITT reactors during the initial bTB herd incident.

Table 1: Descriptive results in relation to future incidents by number of SCITT reactors during the initial bTB incident

Number of SCITT reactors during the initial bTB herd incident	Number of herds	Number of herds with future breakdown	Median time in days to next incident for herds with an incident during the follow-up period	Absolute risk for future breakdown
1	507	160	902	31.6%
2	182	76	803	41.8%
3	91	39	592	42.9%
4	59	26	546	44.1%
5	38	19	586	50.0%
>5	159	88	534	55.3%
Total	1,036	408	708	39.4%

Survival analyses

Visual assessment of the Kaplan-Meier curves shows an increasing risk of future bTB herd incident associated with increasing number of SCITT reactors during the initial bTB herd incident (Figures 2). Confirmation of bTB was marginally associated with an increased risk of future bTB herd incident based on this assessment (Figure 3).

Figure 2: Kaplan Meier curves by number of SCITT reactors during the initial bTB herd incident

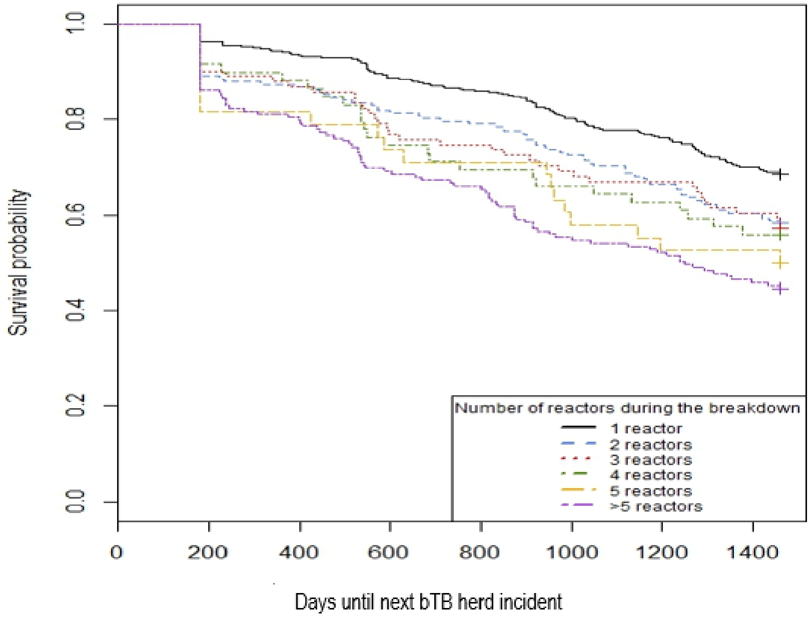
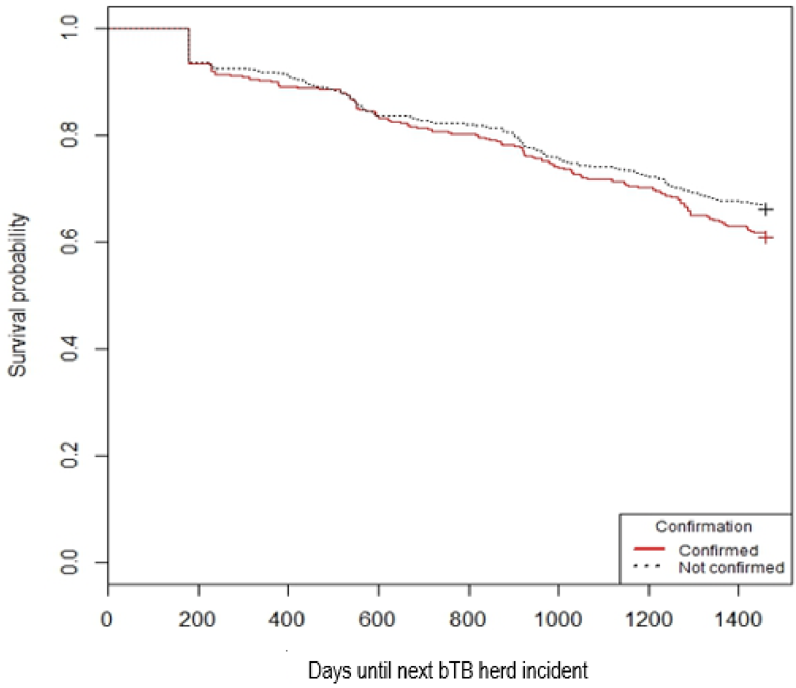


Figure 3: Kaplan Meier curves by bTB confirmation status during the initial bTB herd incident



Cox Regression model

Results for the univariable analyses are displayed in Table 2. Increasing number of SCITT reactors during the initial bTB herd incident and number of SCITT reactors at the disclosure test were associated with a significantly increased risk of a future bTB herd incident (Hazard Ratio=2.300 (95% Confidence Interval (CI) 1.772-2.984) in incidents >5 SCITT reactors compared to incidents with only one SCITT reactor). Confirmation of bTB infection was not significantly associated to risk of future bTB herd incident (Hazard Ratio=1.024; 95% CI 0.834-1.258; *P*=0.821).

Results of the collinearity assessment between variables showed there was a linear correlation between the number of SCITT reactors during the initial bTB herd incident and the number of SCITT reactors at the disclosure test (r = 0.68). The explanatory variable, 'number of SCITT reactors during the bTB herd incident', was therefore used in the multivariable model as it was considered of most biological relevance.

The final multivariable model (Table 3) consisted of the number of SCITT reactors during the bTB herd incident, herd size, bTB history in the previous 3 years, DVO area, herd type, purchase intensity and patch bTB prevalence. Increasing number of SCITT reactors during the bTB herd incident were associated with a significantly increased risk of a future bTB herd incident (Hazard Ratio=1.861 in incidents >5 SCITT reactors compared to incidents with only one SCITT reactor; 95% CI 1.412-2.453; *P*<0.001). Increasing herd size was also significantly associated to risk of future bTB herd incident (Hazard Ratio=1.002 per animal increase; 95% CI 1.001-1.003; *P*<0.001). As were herd type (Hazard Ratio dairy herds versus non-dairy herds=1.271; 95% CI 1.018-1.587; *P*=0.035), purchase intensity (Hazard Ratio = 1.646 (95% CI 1.067-2.539; *P*=0.024) if >33 animals moved into the herd 90 days before the incident in the follow-up period or end of follow-up period) and patch bTB prevalence (Hazard Ratio = 1.761; 95% CI 1.298-2.392; *P*<0.001 for upper quartile compared to lowest quartile). Schoenfeld residuals of the multivariable model showed that the proportional hazard assumption was not violated.

Table 2: Univariable Cox hazard analyses of risk factors for risk of future bTB herd incident

Variable	**Future bTB incident (n=408)**		**No future bTB incident (n=628)**		**Hazard ratio**	**95% CI**	**P Value (Wald test)**
	n	**% cases**	**n**	**% controls**			
Number of SCITT reactors during incident							
1	160	39.2%	347	55.3%	1.000	-	**<0.001**
2	76	18.6%	106	16.9%	1.468	1.118-1.930	
3	39	9.6%	52	8.3%	1.553	1.094-2.203	
4	26	6.4%	33	5.3%	1.575	1.040-2.384	
5	19	4.7%	19	3.0%	1.893	1.177-3.046	
>5	88	21.6%	71	11.3%	2.300	1.772-2.984	
Number of SCITT reactors at disclosure test							
1	195	47.8%	377	60.0%	1.000	-	**<0.001**
2	71	11.5%	96	15.3%	1.350	1.029-1.772	
3	28	6.9%	49	7.8%	1.114	0.750-1.655	
4	27	6.6%	31	4.9%	1.558	1.042-2.330	
5	18	4.4%	16	2.5%	1.733	1.069-2.808	
>5	69	16.9%	59	9.4%	1.965	1.493-2.587	
Herd size							
Per animal increase					1.003	1.002-1.003	**<0.001**
bTB confirmation							
No	136	33.3%	212	33.8%	1.000	-	0.821
Yes	272	66.7%	416	66.25	1.024	0.834-1.258	
Divisional Veterinary Office Area							
Armagh	31	7.6%	50	8.0%	1.000	-	0.104
Ballymena	19	4.7%	37	5.9%	0.861	0.486-1.524	
Coleraine	49	12.0%	76	12.1%	1.038	0.662-1.627	
Dungannon	47	11.5%	75	11.9%	0.998	0.634-1.570	
Enniskillen	57	14.0%	113	18.0%	0.831	0.537-1.287	
Londonderry	15	3.7%	33	5.3%	0.825	0.466-1.460	
Mallusk	19	4.7%	35	5.6%	0.758	0.409-1.403	
Newry	80	19.6%	87	13.9%	1.415	0.934-2.142	
Newtownards	45	11.0%	46	7.3%	1.482	0.938-2.342	
Omagh	46	11.3%	76	12.1%	0.946	0.600-1.492	

Table 2: Continued

Variable	Future bTB incident (n=408)		No future bTB incident (n=628)		Hazard ratio	95% CI	P Value (Wald test)
	n	% cases	n	% controls			
bTB in previous 3 years							
No	251	61.5%	455	72.5%	1.000	-	**<0.001**
Yes	157	38.5%	173	27.5%	1.513	1.239-1.847	
Herd type							
Non-dairy	233	57.1%	479	76.3%	1.000	-	**<0.001**
Dairy	175	42.9%	149	23.7%	1.936	1.591-2.356	
Number of animals moved into the herd 90 days before the bTB herd incident or the end of follow-up period							
0	38	9.3%	101	16.1%	1.000	-	**<0.001**
1-5	58	14.2%	174	27.7%	0.850	0.564-1.279	
6-13	91	22.3%	160	25.5%	1.322	0.905-1.930	
14-33	123	30.1%	127	20.2%	2.106	1.463-3.030	
>33	98	24.0%	66	10.5%	2.895	1.990-4.211	
Patch bTB prevalence							
<Q1	89	21.8%	170	27.1%	1.000	-	**<0.001**
≥Q1 to <Med	91	22.3%	166	26.4%	1.071	0.800-1.434	
≥Med to <Q3	95	23.3%	163	26.0%	1.112	0.833-1.485	
≥Q3	133	32.6%	129	20.5%	1.871	1.430-2.447	

Table 3: Multivariable Cox hazard analyses of defined risk factors for risk of future bTB

Variable	Future bTB incident (N=408)		No future bTB incident (N=628)		Hazard ratio	95% CI	P Value	P Value
	n	**% cases**	**n**	**% controls**				
Number of SCITT reactors during incident								
1	160	39.2%	347	55.3%	1.000	-	**-**	**<0.001**
2	76	18.6%	106	16.9%	1.380	1.045-1.821	**0.023**	
3	39	9.6%	52	8.3%	1.579	1.104-2.257	**0.012**	
4	26	6.4%	33	5.3%	1.461	0.956-2.233	0.080	
5	19	4.7%	19	3.0%	1.585	0.975-2.578	0.063	
>5	88	21.6%	71	11.3%	1.861	1.412-2.453	**<0.001**	
Herd size								
Per animal increase					1.002	1.001-1.003	**<0.001**	**<0.001**
bTB in previous 3 years								
No	251	61.5%	455	72.5%	1.000	-		0.313
Yes	157	38.5%	173	27.5%	1.152	0.931-1.425	0.193	
Divisional Veterinary Office Area								
Armagh	31	7.6%	50	8.0%	1.000	-	-	0.109
Ballymena	19	4.7%	37	5.9%	0.788	0.438-1.418	0.426	
Coleraine	49	12.0%	76	12.1%	1.101	0.690-1.756	0.686	
Dungannon	47	11.5%	75	11.9%	1.361	0.851-2.177	0.198	
Enniskillen	57	14.0%	113	18.0%	0.754	0.477-1.192	0.227	
Londonderry	15	3.7%	33	5.3%	0.799	0.443-1.440	0.456	
Mallusk	19	4.7%	35	5.6%	0.630	0.337-1.178	0.148	
Newry	80	19.6%	87	13.9%	1.444	0.936-2.228	0.097	
Newtownards	45	11.0%	46	7.3%	1.137	0.711-1.820	0.591	
Omagh	46	11.3%	76	12.1%	1.323	0.823-2.124	0.248	
Herd type								
Non-dairy	233	57.1%	479	76.3%	1.000	-	-	**0.021**
Dairy	175	42.9%	149	23.7%	1.271	1.018-1.587	**0.035**	

Table 3: continued

Variable	Future bTB incident (N=408)		No future bTB incident (N=628)		Hazard ratio	95% CI	P Value	P Value
	n	% cases	n	% controls				
Number of animals moved into the herd 90 days before the incident in follow-up period or end of follow-up period								
0	38	9.3%	101	16.1%	1.000	-	-	**<0.001**
1-5	58	14.2%	174	27.7%	0.775	0.513-1.171	0.226	
6-13	91	22.3%	160	25.5%	1.096	0.743-1.615	0.645	
14-33	123	30.1%	127	20.2%	1.402	0.953-2.063	0.087	
>33	98	24.0%	66	10.5%	1.646	1.067-2.539	**0.024**	
Patch bTB prevalence								
<Q1	89	21.8%	170	27.1%	1.000	-	-	**<0.001**
≥Q1 to <Med	91	22.3%	166	26.4%	0.926	0.675-1.269	0.631	
≥Med to <Q3	95	23.3%	166	26.0%	0.910	0.669-1.238	0.549	
≥Q3	133	32.6%	126	20.5%	1.761	1.298-2.392	**<0.001**	

Discussion

Overall, the incidence risk for bTB herd incident for the study herds during the four year follow up was 39.4%, which was similar to the figure obtained in a study carried out in the Republic of Ireland [26]. Additionally, the 4 year risk for a future bTB herd incident almost doubled (Hazard Ratio=1.861) between baseline herds (31.6%; i.e 160/(160+347)*100%) and herds with bTB herd incidents with > 5 SCITT reactors (55.3%; i.e. 88/(88+71)*100%) (see also Table 1). However, bTB confirmation was not predictive of the risk of future bTB herd incidents; a finding supported by several other studies [13-16] and also by the very high specificity of the SCITT [8, 10, 27, 28]. The results in relation to bTB confirmation are similar to previous research conducted in Ireland [13] where in line with the current study bTB confirmation status was non-significantly associated with future bTB incidents in the univariable model and consequently left out of the multivariable model. The other risk factors for bTB recurrence were identified in the current study (herd size, herd-type, animal purchase history and local bTB prevalence) were consistent with previous research studies [reviews by 29 and 30].

There is variation in policy in relation to the control measures applied to bTB herd incidents with unconfirmed bTB infection between different parts of the British Isles. Whereas England's regime tends to differentiate between confirmed and unconfirmed herd incidents with regards to follow up testing except for high risk areas [31], the Republic of Ireland makes very little differentiation and subjects herds with unconfirmed bTB herd incidents to the same follow-up regime as confirmed incidents in nearly all situations, except for herds in which only one bTB reactor is disclosed [32]. Up until 2018, the policy in Northern Ireland was that only herds with more than 5 SCITT reactors and unconfirmed bTB infection were subjected to the same control measures as those with confirmed incidents (OTW regimen). The main reason for evaluating this policy was to identify measures to reduce residual bTB infection in herds.

The sensitivity of the SCITT using Bayesian approaches across the British Isles estimates it to be around 50-60%, depending on different circumstances [10, 27, 28] although previous reviews have suggested higher sensitivity estimates [8]. This indicates that the sensitivity of the SCITT test is moderate at best providing ample opportunity for false negative animals to be left in bTB herd incidents if there is reliance upon one negative SCITT herd test to regain OTF status. Previous studies highlighted the importance of such residual infection in cattle herds [16, 26], which can lead to recurrence [33] alongside the costs associated with further control measures.

In addition to this, the specificity of the SCITT test is estimated to be very high [8-10], which would suggest that the positive predictive value of a herd with multiple SCITT reactors being truly infected with bTB approaches 100% in a country where the infection is endemic [8]. This complements the above logic relating to residual bTB infection in herds. Furthermore, the poor sensitivity of abattoir inspection in finding gross bTB lesions alongside reported variation between abattoirs [34-37] supports the findings from the current study and questions a policy where the follow-up SCITT regime after disclosure of a bTB herd incident is determined by bTB confirmation status.

The main reason why an animal is classified as an unconfirmed SCITT reactor is related to the stage of infection and the techniques employed to identify gross pathology of bTB infection. It is however thought that unconfirmed SCITT reactors could potentially be less likely to shed *M. bovis* than SCITT reactors with visible lesions [38, 2]. Nevertheless, unconfirmed SCITT reactors are known to be able to shed *M. bovis* [1] and the issue of residual infection remains. In line with this, previous research stated that the significance of unconfirmed SCITT reactors depends on the intrinsic specificity of the screening test, the stage of the bTB eradication campaign, thoroughness of examination of reactors at slaughter, time since infection, prevalence of bTB and the number of SCITT reactors found in the herd [12-15]. The latter has been the focus of the current study.

Therefore it can be concluded that herds with multiple SCITT reactors should be subjected to an OTW regimen, irrespective of bTB confirmation. Introduction of this recommended policy change would give rise to greater assurances that herds are free of bTB when they regain OTF status thus limiting the inter-herd dissemination of bTB as well increasing the interval between bTB herd incidents for affected herds. In the longer term, a reduction in bTB herd incidence and overall bTB programme costs may be expected. However, further research to quantify the proportion of bTB infection in herds that is due to recrudescence of infection and that caused by re-infection from animal movements or by local spread is advocated. Nevertheless, the results of this study combined with our understanding of SCITT test performance support such a policy change in the Northern Ireland bTB eradication programme.

Conclusion

The epidemiological evidence presented here demonstrates that the risk of a future bTB herd incident increases directly with the number of SCITT reactors identified during the incident, irrespective of whether bTB infection is confirmed in the herd. The findings indicate that a policy change in relation to control of bTB in herds with multiple SCITT reactors in which bTB has not been confirmed could potentially benefit from application of the same control measures as those applied to confirmed bTB herd incidents.

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Conflict of interest

The authors have no conflicts of interest to declare.

References

1. Menzies FD, Neill SD. Cattle-to-cattle transmission of bovine tuberculosis. *Veterinary Journal* 2000; 160, 92-106

2. Pollock JM, *et al.* Pathogenesis of bovine tuberculosis: the role of experimental models of infection. *Veterinary Microbiology* 2006; 112, 141-150

3. Fenhalls G, *et al. In situ* production of interferon-γ, *interleukin*-4, and tumour necrosis factor α mRNA in human lung tuberculous granulomas. *Infection and Immunity* 2000; 68, 2827-2836

4. Abernethy DA, *et al.* The Northern Ireland programme for the control and eradication of *Mycobacterium bovis. Veterinary Microbiology* 2006; 112, 231-237

5. O'Hagan MJH, *et al.* Risk factors for visible lesions or positive laboratory tests in bovine tuberculosis reactor cattle in Northern Ireland. *Preventive Veterinary Medicine* 2015; 120, 283-290

6. Corner LA. Post mortem diagnosis of *Mycobacterium bovis* infection in cattle. *Veterinary Microbiology* 1994; 40, 53-63

7. Whipple DL, Bolin CA, Miller JM. Distribution of lesions in cattle infected with *Mycobacterium bovis. Journal of Veterinary Diagnostic Investigation* 1996; 8, 351-354

8. De la Rua-Domenech R, *et al.* Ante-mortem diagnosis of tuberculosis in cattle: a review of the tuberculin tests, gamma-interferon assay and other ancillary diagnostic techniques. *Research in Veterinary Science* 2006; 81, 190-210

9. Goodchild AV, *et al.* Specificity of the comparative skin test for bovine tuberculosis in Great Britain. *Veterinary Record* 2015; 177 (258), doi:10.1136/vr.102961

10. Nuñez-Garcia J, *et al.* Meta-analyses of the sensitivity and specificity of ante-mortem and post-mortem diagnostic tests for bovine tuberculosis in the UK and Ireland. *Preventive Veterinary Medicine* 2017; 153, 94-107

11. Karolemeas K, *et al.* Predicting prolonged bovine tuberculosis incidents in Great Britain as an aid to control. *Preventive Veterinary Medicine* 2010; 97, 183-190

12. Olea-Popelka FJ, *et al.* Breakdown severity during a bovine tuberculosis episode as a predictor of future herd incidents in Ireland. *Preventive Veterinary Medicine* 2004; 63, 163-172

13. Wolfe DM, *et al.* From explanation to prediction: a model for recurrent bovine tuberculosis in Irish cattle herds. *Preventive Veterinary Medicine* 2010; 94, 170-177

14. Murray D, Clegg TA, More SJ. Evaluation of single reactor bovine tuberculosis incidents based on analysis of reactors slaughtered at an Irish Export meat plant. *Veterinary Record* 2012; 170, 516-521

15. Doyle LP, *et al.* Bovine tuberculosis in Northern Ireland: risk factors associated with time from post-outbreak test to subsequent herd incident. *Preventive Veterinary Medicine* 2014; 131, 1-7

16. Clegg TA, Good M, More SJ. Future risk of bovine tuberculosis recurrence among higher risk herds in Ireland. *Preventive Veterinary Medicine* 2015; 118, 71-79

17. Houston R. A computerised database system for bovine traceability. *Revenue Scientific et Technique International Office of Epizootics* 2001; 20, 652-661

18. Therneau T. A Package for Survival Analysis in S. version 2.38, https://CRAN.R-project.org/package=survival

19. Doyle LP, *et al.* Bovine tuberculosis in Northern Ireland: risk factors associated with duration and recurrence of chronic herd breakdowns. *Preventive Veterinary Medicine* 2016; 131, 1-7

20. Kaplan EL, Meier P. Non parametric estimation from incomplete observations. *Journal of the American Statistical Association* 1958; 53, 457–481

21. Cox DR, Oakes D. *Analysis of survival data*. Chapman and Hall, London. 1984, pp. 91-111

22. Katz MH. *Multivariable* analysis, a practical guide for clinicians. Cambridge University Press, New York, 2006, pp. 43-47; 137-152

23. Bradburn MJ, *et al.* Survival analysis part III: multivariate data analysis – choosing a model and assessing its adequacy and fit. *British Journal of Cancer* 2003; 18, 605-611

24. Grambsch PM, Therneau TM. Proportional hazards tests and diagnostics based on weighted residuals. *Biometrika* 1994; 81, 515–526

25. Peduzzi P, *et al.* Importance of events per independent variable in proportional hazards regression analyses II. Accuracy and precision of regression estimates. *Journal of Clinical Epidemiology* 1995; 48, 1503-1510

26. Gallagher MJ, *et al.* Comparison of bovine tuberculosis recurrence in Irish herds between 1998 and 2008. *Preventive Veterinary Medicine* 2013; 111, 237-244

27. EFSA. Scientific Opinion on the use of a gamma interferon test for the diagnosis of bovine tuberculosis. EFSA Journal 2012; 10, 2975

28. Clegg TA, *et al*. Using latent class analysis to estimate the test characteristics of the gamma-interferon test, the single intradermal comparative tuberculin test and a multiplex immunoassay under Irish conditions. Veterinary Microbiology 2011; 151, 68-76

29. Skuce RA, Allen AR, McDowell SWJ. Herd-level risk factors for bovine tuberculosis: a literature review. *Veterinary Medicine International* 2012. Published Online: 28 June 2012. doi: 10.1155/2012/621210

30. Broughan JM, *et al.* A review of risk factors for bovine tuberculosis infection in cattle in the UK and Ireland. *Epidemiology and Infection* 2016; 144, 2899-2926

31. TB HUB (http://www.tbhub.co.uk/tb-policy/england/). Accessed 21 November 2017.

32. DAFM (https://www.agriculture.gov.ie/media/migration/animalhealthwelfare/diseasecontrols/tuberculosistbandbrucellosis/diseaseeradicationpolicy/VeterinaryHandbook2017150217.pdf). Accessed 21 November 2017.

33. Good M, *et al.* Impact of the national full herd depopulation policy on the recurrence of bovine tuberculosis in Irish herds, 2003-2005. *Veterinary Record* 2011; 169, 581-585

34. Frankena K, *et al.* Quantification of the relative efficiency of factory surveillance in the disclosure of tuberculosis lesions in attested Irish cattle. *Veterinary Record* 2007; 161, 679-684

35. Olea-Popelka F, *et al.* Relative effectiveness of Irish factories in the surveillance of slaughtered cattle for visible lesions of tuberculosis 2005-2007. *Irish Veterinary Journal* 2012; 65:2. Published Online: 30 January 2012. doi: 10.1186/2046-0481-65-2

36. Wright DM, *et al.* Detectability of bovine TB using the tuberculin skin test does not vary significantly according to pathogen genotype within Northern Ireland. *Infection, Genetics and Evolution* 2013; 19, 15-22

37. Pascual-Linaza AV, *et al.* Efficiency of slaughter house surveillance for the detection of bovine tuberculosis in cattle in Northern Ireland. *Epidemiology and Infection* 2016; 145, 995-1005

38. Pollock JM, Welsh MD, McNair J. Immune responses in bovine tuberculosis: Towards new strategies for the diagnosis and control of disease. *Veterinary Immunology and Immunopathology* 2005; 108, 37-43

Chapter 6

Test Characteristics Estimation of the Tuberculin Skin Test and Post-mortem Examination for Bovine Tuberculosis Diagnosis in Cattle in Northern Ireland Using Bayesian Latent Class Analyses with Adjustments for Co-variates

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Summary

The single intradermal comparative cervical tuberculin (SICCT) test and post-mortem examination are the main diagnostic tools for bovine tuberculosis (bTB) in cattle in the British Isles. Latent class modelling is often used to estimate bTB test characteristics due to the absence of a gold standard. However, the reported sensitivity of especially the SICCT test has shown a lot of variation. We applied both the Hui-Walter latent class model under the Bayesian framework and the Bayesian model specified at the animal level, including various risk factors as predictors, to estimate the SICCT test and post-mortem test characteristics. Data were collected from all cattle slaughtered in abattoirs in Northern Ireland in 2015. Both models showed comparable posterior median estimation for sensitivity of the SICCT test (88.61% and 89.79%, respectively) and for post-mortem examination (53.65% and 52.88%, respectively). Both models showed almost identical posterior median estimates for the specificity (99.99% from both models for SICCT test and 99.66% vs 99.63% for post-mortem examination). However, the animal level model showed much narrower posterior 95% credible intervals, suggesting more certainty for the estimates. It is noteworthy that this study was carried out in slaughtered cattle which may not be representative for the general cattle population.

Keywords: Cattle; *Mycobacterium bovis*; Test Characteristics; Skin test; Post-mortem examination; Latent class model; Bayesian analysis

Introduction

Bovine tuberculosis (bTB) is a chronic, infectious disease caused by *Mycobacterium bovis* that affects cattle and many other mammals including humans worldwide. Infection with this bacterium often remains subclinical for a long period whilst cattle can be infectious. Diagnostics therefore must focus on effective detection of cattle at an early stage of infection [1].

The single intradermal comparative cervical tuberculin (SICCT) test, based on detection of a cell-mediated immune response, is the main ante-mortem diagnostic tool for bTB in cattle in the British Isles [2]. Animals can be classified as reactors to the SICCT test on standard, severe or super-severe interpretation based on the cut-off point used of the measured response to the injected bovine and avian tuberculins into the skin of the neck (the test is carried out defined within the EU Council Directive 64/432/EEC, Annex B). Lowering the cut-off point will increase the sensitivity but in return decrease the specificity of the SICCT test and vice versa [3].

In Northern Ireland all cattle over 6 weeks are tested on an annual basis and positive cattle (reactors) are slaughtered followed by post-mortem examination and laboratory tests [4]. In order to confirm bTB by laboratory tests, most SICCT test reactors with visible lesions (43-60% of reactors animals in Northern Ireland [4]) are subjected to histological examination. Furthermore, those samples that show no histological evidence of bTB are subjected to bacteriological culture as are samples from a proportion of SICCT test reactors without visible lesions [5]. The SICCT test is supplemented by routine abattoir surveillance of all cattle slaughtered aiming to find visible bTB lesions. Due to factors such as the microscopic size of early lesions and the time required to develop a detectable immune response neither the post-mortem examination nor the SICCT test can be expected to detect every infected animal. Furthermore, false negative and false positive reactions to the SICCT test can occur due to a variety of reasons relating to both animal and test related factors including desensitisation, drugs, physiological status, tuberculin used, incorrect testing technique [1] and concurrent infection [6].

The sensitivity of the SICCT test reported in the literature shows a lot of variation and was reported in previous research based on summary values of field trials [1] to be between 52.0 and 100% with median values of 80.0% and 93.5% for standard and severe interpretations respectively. Research based on meta-analyses in a systematic review of the scientific literature using Bayesian logistic regression models concluded the median sensitivity for the SICCT test (standard interpretation) to be 50% with wide Bayesian credible intervals (CrI) (95% CrI 26%, 78% (median sensitivity of 63% (95% CrI 40%, 84%) at severe interpretation) [7]. The same study stated the median sensitivity of routine post-mortem examination at meat inspection to be 71% (95% CrI 37%, 92%).

The specificity of the SICCT test has previously been estimated at over 99.9% [1]. Similar figures were quoted (specificity of 99.98 % (95% Confidence Interval (CI) ±0.004%)) for standard interpretation and 99.91% (95% CI ±0.013%) for severe interpretation [3]. The previously mentioned study using meta-analyses [7] found a median specificity for the SICCT test to be 100% (95% CrI 99%, 100%) and a similar figure for the median specificity of routine post-mortem examination (100%; 95% CrI 99%, 100%).

One of the main problems in relation to determining test characteristics and true disease status is the absence of a gold standard test for bTB. Sensitivity and specificity can be estimated in such cases by using latent class models applying two or more tests to two or more populations with distinct prevalences [8]. However, this approach summarises the test results to the (sub)population level, and it is difficult to include additional evidence available from the data in the analysis. The Bayesian latent class model specified at the animal level [9] offers the possibility of including animal level information such as risk factors for the estimation of test characteristics.

Therefore, although latent class analyses for test characteristic estimation has been conducted previously for bTB diagnostics [10-13], the current study is novel as it aims to address the variation in test characteristic estimates by adding a range of animal level covariates to a Bayesian model in order to provide more precise estimates of the test characteristics for the SICCT test and bTB post-mortem surveillance.

Materials and methods

An observational cohort study encompassing all cattle slaughtered in abattoirs in Northern Ireland in 2015 was conducted. Cattle that were slaughtered but had a presenting herd from outside Northern Ireland were excluded from the analyses.

Data collection and definition of variables

All data were extracted from the Animal and Public Health Information System (APHIS) of the Department of Agriculture, Environment and Rural Affairs (DAERA). Details on all individual cattle, cattle movements and bTB tests conducted since 1988 are stored in this database [14]. Datasets were manipulated using Microsoft Access^{TM} (Microsoft Corporation, USA) and subsequently analysed using R version 3.2.3 (The R Foundation for Statistical Computing) and JAGS version 4.1.0 (http://citeseerx.ist.psu.edu/viewdoc/summary?doi=10.1.1.13.3406).

Data included in the analyses were based on information at animal level and test level. The data presented at animal level included individual measures on breed, sex, age at death, days from last SICCT test to slaughter and last SICCT test reason. The days from the last SICCT test was included in order to adjust for the time difference to slaughter between SICCT test reactors (as they are slaughtered shortly after being disclosed as reactors) and SICCT test negative animals. Breeds were categorized as breeds mainly kept for milk production (dairy) and non-dairy breeds. In relation to sex, three categories were constructed: female, non-castrated male (bull) and castrated male (bullock). Age at death and the duration in days from last SICCT test to slaughter were entered into the model as continuous variables. The last SICCT test reason (i.e. the reason for the last SICCT test being conducted prior to slaughter) was divided into three categories; i.e. routine (in situations where no risk of bTB infection was suspected to be in the herd), at risk (in situations where the herd/animal was at increased risk of having bTB infection) and restricted (in situations where SICCT test reactors or animals with lesions at routine slaughter were found or the herd was at high risk of having bTB infection) [5].

The data presented at test level were based on the test related information of the last SICCT test the animal was subjected to prior to slaughter and the tests after slaughter (including the post mortem inspection result in the abattoir, the histology test and the bacteriological culture test) [15]. The interpretation of the SICCT test was based on recorded measurements of the net bovine rise (NBR), calculated as the increase (in millimetres) at the bovine tuberculin (Lelystad) injection site greater than any increase at the avian tuberculin injection site when measured after 72 hours (as per EU Council Directive 64/432/EEC, Annex B). A standard interpretation is read when the thickness at the site of injection of the bovine antigen is greater than the site of injection of the avian antigen by more than 4 mm. A severe interpretation is one in which the thickness at the bovine site is greater than the avian site by 3-4 mm [13].

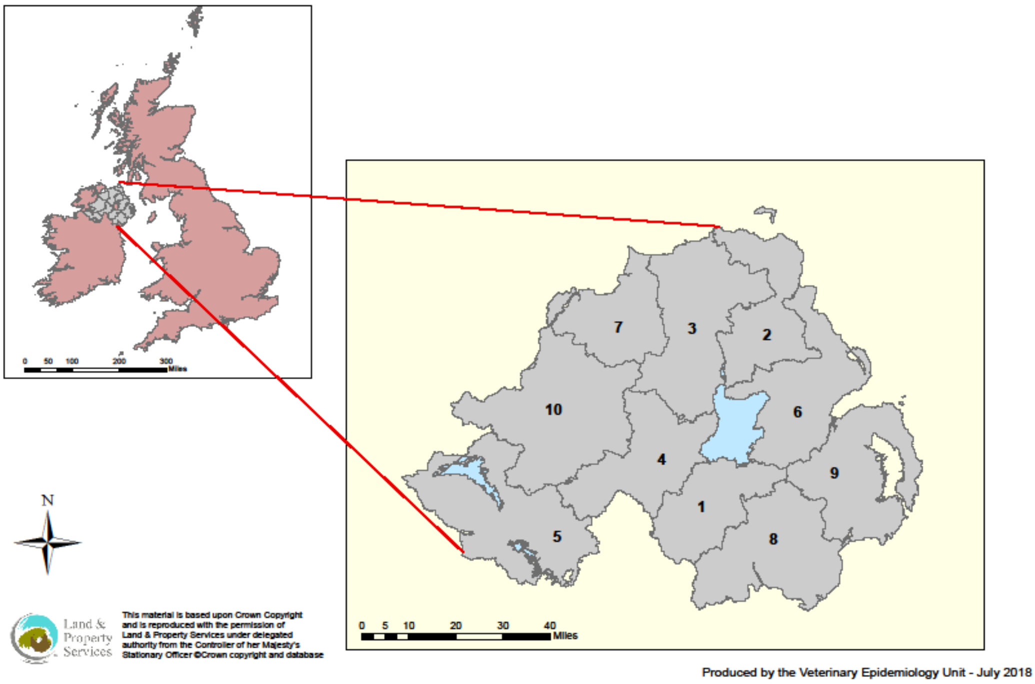
Cattle in the dataset were slaughtered in one of ten abattoirs in 2015. However as practically all SICCT test reactors were slaughtered in one slaughter house (abattoir E), posterior estimates of test characteristics were calculated on both the entire data set and data from animals slaughtered in abattoir E only. Background analysis of lesion distributions between abattoir E and all other abattoirs were conducted in order to assess bias in relation to post-mortem examination techniques between abattoir E and the other abattoirs.

Data analysis

Hui-Walter model

The Bayesian Hui-Walter latent class model [8] was constructed to estimate the test characteristics of SICCT test and post mortem inspection for bTB. The ten Divisional Veterinary Office (DVO) areas were treated as ten subpopulations in Northern Ireland (see Figure 1). Animals were allocated to a DVO area if the last herd they resided in before slaughter was located within this DVO area. We assumed that the ten subpopulations submitted to slaughter had distinct proportions of bTB infected cattle and sensitivity and specificity of the two tests were constant across populations. The two tests were assumed to be independent, conditional on the true disease status of bTB.

Figure 1: Spatial distribution of the ten Divisional Veterinary Office (DVO) areas in Northern Ireland



To check whether the risk factors had an impact on the posterior estimation of sensitivity and specificity for both tests, the Bayesian Hui-Walter model was further applied to stratified samples. Each time the entire dataset (i.e. all cattle slaughtered) was stratified into 2 or 3 samples by one of the five risk factors. The continuous covariates, age at death and days from last SICCT test to slaughter were categorised for the purpose of data stratification. Age at death was divided into two categories, i.e. ≤ 2 years and > 2 years. This cut-off point was used as the majority of cattle bred for meat production are slaughtered by 2 years of age. The duration in days from last SICCT test to slaughter was divided into two categories, i.e. ≤ 45 days and >45 days. This cut-off point was chosen in line with previous research [12].

Non-informative beta prior distributions were specified for the test characteristics (i.e. sensitivity, specificity) and the true proportion of the diseased in each subpopulation.

Animal level model

As can be seen in the Bayesian Hui-Walter approach, stratifying data by a certain risk factor made it possible for us to assess the effect of the risk factor on the estimation of test performance. However, this approach could only investigate one risk factor at a time. In addition, the continuous covariates such as age at death had to be coded into categorical variables prior to data stratification which might cause loss of information.

To estimate the test sensitivity and specificity for SICCT test and post-mortem inspection while taking the possible risk factors into account, a Bayesian logistic regression model was constructed at the animal level [9]. The advantage of this modelling method is that the effect of multiple risk factors can be assessed simultaneously, and continuous covariates can be incorporated without categorisation. In our analysis, the animal level measures on breed, sex, age at death, days from last SICCT test to slaughter and last SICCT test reason were included as risk factors in the logistic regression model (see Appendix A for model code). The categorical variables breed, sex and last SICCT test reason were coded as dummy variables before their addition to the model. Non-informative normal prior distributions were specified for the regression coefficients of the risk factors. Only individual records that consisted of no empty cells from any of the variables mentioned above were used for the analysis. Backward model reduction was performed by comparing the deviance information criterion (DIC) values among the competing models.

For all analyses, four Markov Chain Monte Carlo (MCMC) chains were sampled. Within each chain, the first 5,000 samples were discarded as the burn-in phase, and the subsequent 10,000 samples were used for posterior parameter estimation. Convergence was visually inspected by using trace plots.

Results

Descriptive results

In total 413,383 cattle were slaughtered in abattoirs in Northern Ireland in 2015. A total of 29,839 cattle (7.2%) were dismissed from the analyses due to the fact that their presenting herd was not in Northern Ireland and a further 755 animals (0.2%) had missing values resulting in 382,789 cattle being included in the study.

Bayesian latent class analysis

Hui-Walter model

The posterior medians and 95% credible intervals (CrI) obtained for the test sensitivity and specificity of the SICCT test (standard/severe interpretation) and post-mortem inspection are listed in Table 1 along with the estimated proportion of diseased in the subpopulations (i.e. 10 DVO areas). When the standard interpretation was used for the SICCT test, the estimated sensitivity (%) for the SICCT test was 88.61 [95% CrI 85.39 – 92.23] and the estimated sensitivity for the post-mortem inspection was 53.65 [95% CrI 52.59 – 54.75]. The estimated specificities (%) for both tests were very high (99.99 for the SICCT test and 99.66 for the post-mortem inspection, respectively). Further, as expected, when the cut-off point was changed from the standard to severe interpretation, the sensitivity for the SICCT became higher (93.27; 95% CrI 90.15 – 96.55] while that for post-mortem inspection fell slightly (50.87, 95% CrI 49.88 – 51.92]. However, the specificity remained very high for both tests.

Table 1: Posterior estimates (median and CrI) for SICCT test (standard/severe interpretation of the variable *Net bovine rise*), post mortem examination characteristics and proportions of the diseased in the subpopulations (DVO areas) based on the entire dataset

	Standard interpretation (based on *Net bovine rise*)	Severe interpretation (based on *Net bovine rise*)
Sensitivity SICCT test (%)	88.61 [85.39 – 92.23]	93.27 [90.15 – 96.55]
Specificity SICCT test (%)	99.99 [99.97 – 100.00]	99.99 [99.96 – 100.00]
Sensitivity post-mortem (%)	53.65 [52.59 – 54.75]	50.87 [49.88 – 51.92]
Specificity post-mortem (%)	99.66 [99.60 – 99.71]	99.68 [99.62 – 99.73]
DVO 1 (%)	1.55 [1.43 – 1.68]	1.68 [1.56 – 1.81]
DVO 2 (%)	1.51 [1.34 – 1.69]	1.63 [1.45 – 1.81]
DVO 3 (%)	2.47 [2.29 – 2.66]	2.63 [2.45 – 2.81]
DVO 4 (%)	1.76 [1.63 – 1.89]	1.89 [1.76 – 2.03]
DVO 5 (%)	7.30 [6.83 – 7.78]	7.85 [7.38 – 8.34]
DVO 6 (%)	1.12 [0.99 – 1.27]	1.33 [1.18 – 1.48]
DVO 7 (%)	2.47 [2.20 – 2.76]	2.59 [2.33 – 2.88]
DVO 8 (%)	2.98 [2.79 – 3.16]	3.16 [2.97 – 3.34]
DVO 9 (%)	3.93 [3.66 – 4.18]	4.22 [3.97 – 4.49]
DVO 10 (%)	3.56 [3.34 – 3.77]	3.72 [3.51 – 3.93]

Posterior parameter estimates based on stratified samples are presented in Table 2. For the stratified sample that contained only animals that were sent to slaughter after 45 days from their last SICCT test, due to too few SICCT test reactors, the test sensitivity and specificity of the tests could not be estimated.

Table 2: Posterior estimates (median and 95% CrI) for the sensitivity and specificity for SICCT test (standard interpretation of the variable *Net bovine rise*) and post-mortem examination derived from the stratified population based on risk factors

Stratified population	SICCT test		Post-mortem	
	Sensitivity (%)	Specificity (%)	Sensitivity (%)	Specificity (%)
Age at death				
≤ 2 years	91.95 [85.92 – 98.57]	99.97 [99.88 – 100.00]	60.42 [58.40 – 63.07]	99.71 [99.60 – 99.82]
> 2 years	86.75 [82.80 – 90.74]	99.99 [99.97 – 100.00]	50.29 [48.98 – 51.67]	99.63 [99.56 – 99.69]
Days from last SICCT test to slaughter				
≤ 45 days	90.30 [87.84 – 92.84]	99.98 [99.89 – 100.00]	53.68 [52.64 – 54.82]	99.69 [99.53 – 99.84]
> 45 days	--	--	--	--
Breed				
Dairy	89.85 [86.10 – 93.64]	99.99 [99.94 – 100.00]	47.32 [45.79 – 48.96]	99.70 [99.61 – 99.79]
Non-dairy	89.85 [84.80 – 94.98]	99.99 [99.94 – 100.00]	59.25 [57.67 – 61.11]	99.61 [99.55 – 99.68]
Sex				
Bull	93.33 [80.03 – 99.66]	99.95 [99.76 – 100.00]	30.94 [25.99 – 36.15]	99.66 [99.47 – 99.86]
Bullock	72.23 [62.39 – 83.65]	99.98 [99.89 – 100.00]	63.75 [60.31 – 70.89]	99.82 [99.72 – 99.93]
Female	90.77 [87.83 – 93.70]	99.99 [99.96 – 100.00]	52.92 [51.71 – 54.16]	99.57 [99.50 – 99.65]
Last SICCT *test reason*				
Routine	93.85 [84.75 – 99.55]	99.98 [99.91 – 100.00]	56.56 [53.11 – 61.19]	99.67 [99.62 – 99.74]
Restricted	84.51 [80.43 – 88.65]	99.99 [99.96 – 100.00]	49.50 [48.05 – 50.98]	99.67 [99.58 – 99.76]
Risk	93.27 [88.99 – 97.41]	99.96 [99.87 – 100.00]	59.73 [57.71 – 62.29]	99.60 [99.51 – 99.69]

Animal level model

The majority of SICCT reactors (8956 out of in total 8963 (99.9%)) was sent to Abattoir E. Therefore, the animal level model with risk factors was performed on Abattoir E data only. The standard interpretation of the NBR was used for all subsequent analyses. Potential bias in post-mortem techniques employed between abattoir E and other abattoirs was assessed by means of investigating the lesion distribution in relation to count of sites, locations, number, nature and size of the lesions for SICCT test reactors and non-reactors. No significant difference was detected between SICCT test reactors and non-reactors regarding the number, nature and size of the lesions (See Appendix B).

Table 3 presents the distribution of test results from the SICCT test and post-mortem inspection from the samples stratified by the risk factors within Abattoir E. The risk factors age at death and days from last SICCT test to slaughter are shown as categorical variables to provide an overview of the test positive and negative counts from both tests (Table 3). In the model where risk factors were incorporated, these two covariates remained continuous and were not coded into categorical variables.

Table 4 presents the posterior parameter estimates from the best fitting (i.e. lowest DIC) animal level model and the effect of the risk factors on the odd ratios calculated from the regression coefficients for the risk factors. Posterior estimates from the Hui-Walter model that aggregated both test results to a cross tabulation at the DVO level for Abattoir E are also listed (Table 4).

The results showed that increasing age at death and days from last SICCT test to slaughter were negatively correlated to the odds of bTB infection. Furthermore, compared to bulls, females and bullocks had a smaller odds of bTB infection. The odds of being disclosed with bTB was higher for animals whose last SICCT test prior to slaughter was a ‘routine’ test or a ‘risk’ test compared to animals being subjected to a ‘restricted’ test.

Table 3: Descriptive statistics of the relationship between risk factors and SICCT test and post-mortem examination results from Abattoir E

Covariate	SICCT test positive			Post-mortem positive		
	Yes	No	% of positives	Yes	No	% of positives
Age at death						
≤ 2 years	3,174	12,446	20.3	2,048	13,572	13.1
> 2 years	5,777	36,438	13.7	3,400	38,815	8.1
Days from last SICCT test to slaughter						
≤ 45 days	8,944	14,573	38.0	5,312	18,205	22.6
> 45 days	7	34,311	0.02	136	34,182	0.40
Breed						
Dairy	4,063	17,736	18.6	2,205	19,594	10.1
Non-dairy	4,888	31,148	13.6	3,243	32,793	9.0
Sex						
Bull	331	903	26.8	109	1,125	8.8
Bullock	1,506	18,934	7.4	1,029	19,411	5.0
Female	7,114	29,047	19.7	4,310	31,851	11.9
Last SICCT test reason						
Routine	1,219	14,300	7.9	747	14,772	4.8
Restricted	4,601	20,323	18.5	2,669	22,255	10.7
Risk	3,131	14,261	18.0	2,032	15,360	11.7

Table 4: Posterior estimates (median and 95% CrI) from the Hui-Walter model and the best fitting animal level model with risk factors using Abattoir E data

	Hui-Walter model	
	SICCT test	Post-mortem
Sensitivity (%)	92.07 [90.84 – 93.30]	53.60 [52.58 – 54.65]
Specificity (%)	99.98 [99.94 – 100.00]	99.49 [99.36 – 99.62]

	Animal level model with risk factors	
	SICCT test	Post-mortem
Sensitivity (%)	89.79 [89.27 – 90.58]	52.88 [52.13 – 53.49]
Specificity (%)	99.999 [99.996 – 100.00]	99.63 [99.59 – 99.67]

	Effect of risk factors (odds ratio)
Age at death (per day increase)	0.9994 [0.9992 – 0.9996]
Days from last SICCT test to slaughter (per day increase)	0.890 [0.888 – 0.892]
Sex	
Bull	Reference category
Bullock	0.157 [0.144 – 0.178]
Female	0.463 [0.429 – 0.526]
Last SICCT test reason	
Restricted	Reference category
Routine	1.518 [1.424 – 1.605]
Risk	1.809 [1.717 – 1.903]

Discussion

Estimation of test characteristics for diagnostic tests in the absence of a gold standard is notoriously difficult and has been reflected by the increased use of Bayesian latent class analyses to overcome this problem [16]. In the case of bTB diagnostics, in the absence of an accurate reference standard, these analyses have been used previously in order to calculate diagnostic test characteristics [10-13].

Nevertheless, even with the use of Bayesian latent class modelling, a lot of variation especially in relation to sensitivity estimates of ante- and post-mortem tests for bTB have been reported [7]. The current study aimed to apply a method to obtain more accurate estimates especially in relation to the test sensitivity by adding risk factors measured at the animal level.

By choosing two populations in order to conduct the latent class analyses one of the issues in relation to bTB is that only cattle positive for the SICCT test will have an 'immediate' post-mortem result available. In order to have the availability of post-mortem results in the entire study population it was decided to choose the study population as 'all cattle slaughtered in 2015'. This approach means that all cattle that were recorded as reactors to the SICCT test in 2015 were in the study population but not all cattle that were negative to the SICCT test. One of the consequences of this is that the prevalence calculated in the sub populations (DVO areas – Table 1) do not reflect the prevalence in the actual DVO areas as a whole; it merely represents the bTB prevalence of all the cattle slaughtered presented by herds within these DVO areas. However, these calculated prevalences indicate that the ten DVO areas have distinct bTB proportions which is one of the prerequisites for latent class modelling [8].

The specificity estimated for both the SICCT test and post-mortem examination were very high (>99.4%) in all analyses (i.e. all cattle slaughtered, abattoir E only, standard and severe interpretation of the SICCT test, with and without addition of animal level risk factors) with narrow credibility intervals. These estimates are similar to previous estimates [7] and show that both bTB diagnostic tests are very unlikely to report a false positive animal.

The sensitivity estimates for the SICCT test (varying from 88.61 (standard interpretation) to 93.27 (severe interpretation) were on the high end of figures reported by previous studies. The chosen population (i.e. all animals slaughtered in 2015) is potentially creating a bias for SICCT test reactors compared to SICCT test negative animals as SICCT test reactors are always slaughtered whereas SICCT test negative animals are not. Furthermore, previous research conducted in Northern Ireland [13] (reported sensitivity at standard interpretation 40.5-57.7) was based on chronic bTB breakdown herds only suggesting that these are herds that are tested on short intervals for prolonged periods of time driving the sensitivity of the SICCT test down. Moreover, estimates for chronic bTB breakdown herds used gamma interferon test results for their analyses creating a bias towards herds that have already been censored through removal of SICCT test reactors during at least one previous recent herd SICCT test. The sensitivity estimates reported previously in the Republic of Ireland [10] were lower as well, whereas our estimates were more in line with previously reported figures from England [11]. This is potentially due to the difference in bTB prevalence in the cattle population [17] and the stage and details of the bTB eradication programmes showing more similarities between England and Northern Ireland than between Ireland and Northern Ireland [18]. However, it has to be noted that the current study was carried out in slaughtered cattle in Northern Ireland which may not be representative for the general cattle population.

The estimated sensitivity of post-mortem examination was similar to previous reported figures [7]. It was noteworthy that when severe interpretation was applied, lower sensitivity for post-mortem examination was obtained (Table 1). This might be due to the increased false negatives caused by the severe interpretation from the SICCT test, which affected the cross tabulation of the joint test results from SICCT test and post-mortem inspection for the Bayesian Hui-Walter latent class model. Furthermore, severe interpretation is usually applied in herds when there is already infection in the herd and the herd therefore has been tested recently. It follows that lesions would not have had the time to develop to the 'visible' stage in terms of post-mortem inspection. Analyses based on data for abattoir E only were conducted in order to account for potential differences in the post-mortem examinations conducted between abattoir E and the other abattoirs based on the fact that abattoir E was the destination for practically all (99.9%) SICCT test reactors. Distributions of post-mortem lesions by number, nature and size showed no differences between SICCT test reactors and those detected by post-mortem inspection only. This was backed up by the current study finding no significant differences in estimated test characteristics between abattoir E and the rest of the abattoirs (data not shown).

The estimates for the sensitivity and specificity of the tests varied among the stratified samples (Table 2), indicating that the parameter estimation was affected by the risk factors. Comparing the test characteristic results between the Hui-Walter model and the animal level model including the risk factors age at death, days from last test to slaughter, sex and last SICCT test reason showed that adding the risk factors created smaller 95% credible intervals (Table 1 versus Table 4). This suggests that by adding animal level risk factors, we included more information to estimate the sensitivity and specificity for the SICCT test and post-mortem examination.

Furthermore, the relationship between each risk factor and the true disease status was assessed and quantified. The model evaluated possible risk factors for the bTB infection status of the animal as detected by these two diagnostic tests within the population of cattle slaughtered in 2015. The best fitting model indicated that the risk of having a positive bTB status was significantly influenced by the animal level characteristics age at death, days from last SICCT test to slaughter, sex and last SICCT test reason.

Age at death and days from last SICCT test to slaughter were negatively correlated to the odds of bTB infection, namely animals with an older age or a longer duration in days from the last SICCT test to slaughter were indicated to have lower odds (0.9994 and 0.890 per day, respectively). Increasing age is a risk factor for bTB breakdown [19], but similarly it is protective in relation to the development of visible lesions [5, 20]. The finding that longer duration in days from the last SICCT test to slaughter was indicated as being protective to finding a positive bTB status is as expected as the median time for removal of bTB reactors in 2015 was 8.9 working days [21]. It follows that all SICCT test reactor animals had a shorter time interval between their last SICCT test before slaughter compared to the SICCT test negative animals in the data set.

Furthermore, compared to bulls, female and castrated male animals (bullocks) tended to have smaller odds of bTB infection detection (0.46 and 0.16, respectively). Sex was not shown to be a risk factor for bTB infection, once adjusted for age, in studies previously conducted in the Republic of Ireland [22], but SICCT test positive bulls were shown to be less likely to develop visible lesions [5]. Relative differences in bTB disclosure in relation to sex may be masked by differences in longevity of beef and dairy cattle and different ‘between and within’ herd movements and contacts experienced [19].

The odds of bTB infection was estimated to be 1.52 times higher for animals that were subjected to a ‘routine’ test prior to slaughter than for animals that were subjected to a ‘restricted’ test (reference category), whereas the odds of bTB infection for animals subjected to a ‘risk’ test prior to slaughter was estimated to be 1.81 times higher than the animals subjected to a ‘restricted’ test. This was only the case when the model included the risk factor days from the last SICCT test to slaughter. If the risk factor last SICCT test reason was included as the only covariate for the Bayesian animal level latent class model, the odds of bTB infection detection for ‘routine’ farms was the lowest and the odds for ‘restricted’ farms was the highest (results not shown) as one would expect. It is worth noting that 58.8% slaughtered cattle that had ≤ 45 days from last test to slaughter at Abattoir E were from the ‘restricted’ farms, and 61.0% when all abattoirs were included. Furthermore, no significant relationship was found between the odds of bTB infection and the animal level characteristic breed.

It should be noted that as our study was carried out in slaughtered cattle in Northern Ireland, the estimated effect of the risk factors on the odds of bTB infection may not be representative for the general cattle population (1.75 million cattle). Further research may adapt this model to the general population.

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Conflict of interest

The authors have no conflicts of interest to declare.

References

1. De la Rua-Domenech R, et al. Ante-mortem diagnosis of tuberculosis in cattle: a review of the tuberculin tests, gamma-interferon assay and other ancillary diagnostic techniques. Research in Veterinary Science 2006; 81, 190-210

2. Pollock JM et al. Specific delayed-type hypersensitivity responses to ESAT-6 identify tuberculosis-infected cattle. Journal of Clinical Microbiology 2003; 41, 1856-1860

3. Goodchild AV, et al. Specificity of the comparative skin test for bovine tuberculosis in Great Britain. Veterinary Record 2015; 177, 258-267

4. Abernethy DA, et al. The Northern Ireland programme for the control and eradication of Mycobacterium bovis. Veterinary Microbiology 2006; 112, 231-237

5. O'Hagan MJH et al. Risk factors for visible lesions or positive laboratory tests in bovine tuberculosis reactor cattle in Northern Ireland. Preventive Veterinary Medicine 2015; 120, 283-290

6. Godfray et al. A restatement of the natural science evidence base relevant to the control of bovine tuberculosis in Great Britain. Proceedings of the Royal Society B: Biological Sciences 2013; 280: 20131634

7. Nuñez-Garcia J et al. Meta-analyses of the sensitivity and specificity of ante-mortem and post-mortem diagnostic tests for bovine tuberculosis in the UK and Ireland. Preventive Veterinary Medicine 2017; 153, 94-107

8. Hui SL, Walter SD. Estimating the error rates of diagnostic tests. Biometrics 1980; 36, 167-171

9. Koop G, et al. Risk factors for subclinical intramammary infection in dairy goats in two longitudinal field studies evaluated by Bayesian logistic regression. Preventive Veterinary Medicine 2013; 108, 304-312

10. Clegg TA, et al. Using latent class analyses to estimate the test characteristics of the γ-interferon test, the single intradermal comparative tuberculin test and a multiplex immunoassay under Irish conditions. Veterinary Microbiology 2011; 151, 86-76

11. Karolemeas K, et al. Estimation of relative sensitivity of the comparative tuberculin skin test in tuberculous cattle herds subjected to depopulation. PLoS ONE 2012; 7, e43217

12. Bermingham ML, et al. Hui and Walter's latent-class model extended to estimate diagnostic test properties from surveillance data: a latent model for latent data. Scientific Reports 2015; 5, 11861; doi: 10.1038/srep11861

13. Lahuerta-Marin A, et al. Bayesian latent class estimation of sensitivity and specificity parameters of diagnostic tests for bovine tuberculosis in chronically infected herds in Northern Ireland. Veterinary Journal 2018; 238, 15-21

14. Houston R, et al. A computerised database system for bovine traceability. Revenue Scientifique et Technique, International Office of Epizootics 2001; 20, 652-661

15. OIE Terrestrial Manual 2009. Chapter 2·4·7. On line available on: (http://www.oie.int/fileadmin/Home/eng/Health_standards/tahm/2008/pdf/2.04.07_BOVINE_TB.pdf). Accessed 10 September 2018

16. Van Smeden M, et al. Latent class models in diagnostic studies when there is no reference standard – a systematic review. American Journal of Epidemiology 2014; 179, 423-431

17. Brenner H, Gefeller O. Variation of sensitivity, specificity, likelihood ratios and predictive values with disease prevalence. Statistics in Medicine 1997; 16, 981-991

18. Abernethy DA, et al. Bovine tuberculosis trends in the UK and the republic of Ireland, 1995-2010. Veterinary Record 2013; 172, 312

19. Skuce RA, Allen AR, McDowell SWJ. Bovine Tuberculosis (TB): A review of cattle-to-cattle transmission, risk factors and susceptibility, 2011. On line available on: https://www.daera-ni.gov.uk/sites/default/files/publications/dard/afbi-literature-review-tb-review-cattle-to-cattle-transmission.pdf Accessed 14th August 2018

20. Byrne AW, et al. Bovine tuberculosis visible lesions in cattle culled during herd breakdowns: the effects of individual characteristics, trade movement and co-infection. BMC Veterinary Research 2017; 13:400, Doi 10.1186/s12917-017-1321-z

21. DAERA. Department of Agriculture, Environment and Rural Affairs, Bovine Tuberculosis statistics 2015. On line available on: https://www.daera-ni.gov.uk/publications/tuberculosis-disease-statistics-northern-ireland-2015 . Accessed on 14th August 2018

22. Clegg TA et al. Potential infection-control benefit for Ireland from pre-movement testing of cattle for tuberculosis. Preventive Veterinary Medicine 2008; 84, 94-111

Appendix A

Population level model

```
model{



for (j in 1:nr.pops) {

p[j] ~ dbeta(1,1)

pop[j, 1:4] ~ dmulti(par[j,1:4], n[j])
par[j, 1] <- p[j]*Se1*Se2 + (1-p[j])*(1-Sp1)*(1-Sp2)
par[j, 2] <- p[j]*Se1*(1-Se2) + (1-p[j])*(1-Sp1)*Sp2
par[j, 3] <- p[j]*(1-Se1)*Se2 + (1-p[j])*Sp1*(1-Sp2)
par[j, 4] <- p[j]*(1-Se1)*(1-Se2) + (1-p[j])*Sp1*Sp2

}

## priors
Se1 ~ dbeta(1, 1)
Sp1 ~ dbeta(1, 1)
Se2 ~ dbeta(1, 1)
Sp2 ~ dbeta(1, 1)



}
```

Best fitting model with animal level covariates

```
data {

for (i in 1:cum.dvo10) {
ones[i] <- 1

}
}



model{


## modelling the data on animal level
for (i in 1:cum.dvo10) {

#pop[i, 1:4] ~ dmulti(par[i, 1:4], 1)
par[i, 1] <- pi[i]*Se1*Se2 + (1-pi[i])*(1-Sp1)*(1-Sp2)
par[i, 2] <- pi[i]*Se1*(1-Se2) + (1-pi[i])*(1-Sp1)*Sp2
par[i, 3] <- pi[i]*(1-Se1)*Se2 + (1-pi[i])*Sp1*(1-Sp2)
par[i, 4] <- pi[i]*(1-Se1)*(1-Se2) + (1-pi[i])*Sp1*Sp2


## Define/compute the contribution to the likelihood from the ith observation
L[i]<- equals(tests[i, 1], 1)*equals(tests[i, 2], 1)*par[i, 1]
+ equals(tests[i, 1], 1)*equals(tests[i, 2], 0)*par[i, 2]
+ equals(tests[i, 1], 0)*equals(tests[i, 2], 1)*par[i, 3]
+ equals(tests[i, 1], 0)*equals(tests[i, 2], 0)*par[i, 4]

## incorporate animal level variables
logit(pi[i]) <- beta_0 + beta_1 * death.age[i] + beta_2 * days.bfslau[i] + beta_3 * Female[i] +
beta_4 * Male[i] + beta_5 * Risk[i] + beta_6 * Routine[i]

p[i] <- L[i] / 1
ones[i] ~ dbern(p[i])
}
```

```
## prior
Se1 ~  dbeta(1, 1)
Sp1 ~  dbeta(1, 1)
Se2 ~  dbeta(1, 1)
Sp2 ~  dbeta(1, 1)

beta_0 ~ dnorm(0, 0.001)
beta_1 ~ dnorm(0, 0.001)
beta_2 ~ dnorm(0, 0.001)
beta_3 ~ dnorm(0, 0.001)
beta_4 ~ dnorm(0, 0.001)
beta_5 ~ dnorm(0, 0.001)
beta_6 ~ dnorm(0, 0.001)



## mean of the animal level predicted probabilities for bTB per subpopulation
mean.pi1<-mean(pi[1:cum.dvo1])
mean.pi2<-mean(pi[(cum.dvo1+1):cum.dvo2])
mean.pi3<-mean(pi[(cum.dvo2+1):cum.dvo3])
mean.pi4<-mean(pi[(cum.dvo3+1):cum.dvo4])
mean.pi5<-mean(pi[(cum.dvo4+1):cum.dvo5])
mean.pi6<-mean(pi[(cum.dvo5+1):cum.dvo6])
mean.pi7<-mean(pi[(cum.dvo6+1):cum.dvo7])
mean.pi8<-mean(pi[(cum.dvo7+1):cum.dvo8])
mean.pi9<-mean(pi[(cum.dvo8+1):cum.dvo9])
mean.pi10<-mean(pi[(cum.dvo9+1):cum.dvo10])

}
```

Appendix B

Table S1: Description of number (%) of animals with lesions at a particular location, median number of sites per animal having lesion (1-9 with 9 referring to ≥ 9), nature (mode; 1=calcified; 2=purulent; 3=caseous) and size (median; 1-10 mm with 10 referring to ≥ 10mm) of the lesions for SICCT test reactors and non-reactors with positive post mortem results.

Location	SICCT positive				SICCT negative			
	Number (%) of animals with lesions	Number of sites (median)	Nature (mode)	Size (median)	Number (%) of animals with lesions	Number of sites (median)	Nature (mode)	Size (median)
All sites	5214 (100%)				288 (100%)			
Bronchio mediastinal	3653 (70.06%)	9	1	10	205 (71.18%)	9		9
Head	1697 (32.55%)	9	1	10	86 (29.86)	9		10
Mesenterium	370 (7.10%)	2	1	10	5 (1.74%)	2	3	3
Lungs	214 (4.10%)	9	1	10	34 (11.81%)	9		2
Prescapular left	68 (1.30%)	9	1	10	0 (0.00%)	-	-	-
Liver	45 (0.86%)	4	1	10	14 (4.86%)	9		10
Pleura	43 (0.82%)	9	1	10	5 (1.74%)	9		10
Precrural 1	19 (0.36%)	2	1	10	0 (0.00%)	-	-	-
Prescapular right	13 (0.25%)	9	3	10	2 (0.69%)	5.50	2	10
Peritoneum	9 (0.17%)	9	1	10	3 (1.04%)	9	1	10
Kidney 1	6 (0.11%)	5.50	1	6.50	0 (0.00%)	-	-	-
Ham2	5 (0.10%)	2	3	10	0 (0.00%)	-	-	-
Submammarian	4 (0.08%)	5.50	3	7.5	0 (0.00%)	-	-	-
Popliteal 1	3 (0.06%)	1	3	10	1 (0.35%)	3	3	3
Forequarter	3 (0.06%)	6	3	10	0 (0.00%)	-	-	-
Kidney 2	2 (0.04%)	9	1	10	0 (0.00%)	-	-	-
Ham	2 (0.04%)	1.50	3	6.50	0 (0.00%)	-	-	-
Precrural 2	2 (0.04%)	9.50	1 or 3	9.50	0 (0.00%)	-	-	-
All gut	1 (0.02%)	9	1	10	1 (0.35%)	9	1	10
Spleen	1 (0.02%)	1	1	4	0 (0.00%)	-	-	-
Popliteal 2	1 (0.02%)	9	1	10	0 (0.00%)	-	-	-
Foreleg	1 (0.02%)	1	1	10	0 (0.00%)	-	-	-
Pelvis	1 (0.02%)	9	1	10	0 (0.00%)	-	-	-
Retropharyngeal	0 (0.00%)	-	-	-	5 (1.74%)	10		10
Submaxiliar	0 (0.00%)	-	-	-	0 (0.00%)	-	-	-
All pluck	0 (0.00%)	-	-	-	0 (0.00%)	-	-	-
Diaphragm	0 (0.00%)	-	-	-	0 (0.00%)	-	-	-
Neck	0 (0.00%)	-	-	-	0 (0.00%)	-	-	-
Sternum	0 (0.00%)	-	-	-	0 (0.00%)	-	-	-
All offal	0 (0.00%)	-	-	-	0 (0.00%)	-	-	-
Stomach	0 (0.00%)	-	-	-	0 (0.00%)	-	-	-
Inguinal	0 (0.00%)	-	-	-	0 (0.00%)	-	-	-
Skin	0 (0.00%)	-	-	-	0 (0.00%)	-	-	-
Hindleg	0 (0.00%)	-	-	-	0 (0.00%)	-	-	-
Tail	0 (0.00%)	-	-	-	0 (0.00%)	-	-	-

Chapter 7

General Discussion

The importance of bovine tuberculosis in Northern Ireland

Northern Ireland covers an area of 14,130 km², of which 46.4% is rural (European Commission; 2014). In 2017 there were approximately 1.7 million cattle in Northern Ireland (unchanged from the previous year) distributed among approximately 24,000 active herds with the predominant herd type being beef-cow herds. In 2017 there were approximately 315,800 dairy cows and 267,100 beef cows. Although some large herds are present, the majority of farms are relatively small. Sixty-six per cent of dairy cows are in herds of 100 or more cows, compared with 9 per cent of beef cows. The dairy and beef industry is responsible for approximately 50% of the gross output of the agricultural sector in Northern Ireland (Department of Agriculture, Environment and Rural Affairs, 2017). The agri-food sector in Northern Ireland is much more important to the local economy than is the case for other parts of the United Kingdom. The sector therefore holds an important trade position which, particular in light of Brexit, highlights the need for control/eradication of animal diseases such as bovine tuberculosis (bTB) (TB Strategic Partnership Group, 2016).

Bovine TB has a global distribution and also affects several European countries with highly developed cattle industries and statutory eradication programmes. The disease has implications at a variety of levels including livestock agriculture, wildlife ecology and public health (Robinson, 2014). For Northern Ireland specifically, the European Union (EU) approved bovine tuberculosis eradication programme (EU Trade Directive 64/432/EEC as amended) enables them to trade internationally which is of crucial importance to the Northern Ireland export dependent agri-food sector which is worth about £1.351 billion per year in sales outside the United Kingdom. Current EU rules mean that a herd has to be officially free from bTB before live animals can be traded within Northern Ireland as well as elsewhere. Furthermore milk from bTB reactors has to be excluded from the food chain with the milk from the rest of the herd having to be appropriately heat treated. Meat for export has to be certified as being free from generalised bTB and fit for consumption (TB Strategic Partnership Group, 2016). Furthermore, in relation to trade, the eradication programme reduces the risk of disease in humans and clinical disease in cattle. On the flip side of this it is important to realise that the eradication programme in Northern Ireland costs approximately £40 million for the 2017/2018 financial year (R.

Harwood; personal communication) which does not include the estimated additional cost to the farming industry. Although the previously made points refer to monetary impacts, there are important psychological impacts that play a role as well including the stress of testing, animal movement restrictions and losing cattle (at times based on cattle breeding lines built up over generations) (Robinson, 2014).

Obstacles in relation to bovine tuberculosis eradication

Bovine tuberculosis eradication in Northern Ireland began in 1935 with the slaughter of clinically infected cattle followed by the initiation of a compulsory bTB control programme in 1959. Initially significant progress was made but since the mid-1960s disease has fluctuated without any real improvement (Abernethy et al., 2006; Abernethy et al., 2013). This persistence of bTB infection occurring in cattle herds in Northern Ireland is either due to residual infection (e.g. due to infected cattle not being disclosed by the implemented diagnostic test) or recurrent infection (e.g. following cattle introduction) or local persistence (such as spread from the environment, wildlife or neighbouring farms) (More and Good, 2015).

There are a variety of reasons for this persistence of infection and therefore the lack of success of bTB eradication including:

- the imperfect sensitivity of the currently available diagnostic tests leading to infected animals being left behind in a herd
- the little sense of ownership by farmers resulting in the farming community seeing bTB as a disease for the Government to address which in turn leads to lack of pro-active steps to try and control bTB by the farmers themselves
- the nature of the farming industry in Northern Ireland including high herd and cattle density, small farm unit size, extensive use of outlying, rented pasture, significant between-herd movement and winter housing of cattle
- the presence of a wildlife reservoir (European badger, *Meles meles*)

Imperfect sensitivity of diagnostic tests

Clinical signs of bTB (i.e. coughing and weight loss) are now rarely seen in the United Kingdom due to the slow progression of disease and the Government's compulsory testing and slaughter programme. This rules out reliance of bTB disclosure on a diagnosis based on clinical symptoms. Surveillance for bTB in Northern Ireland therefore is based on a combination of ante- and post-mortem tests as described in the introduction of this thesis.

The SICCT test is the main ante-mortem diagnostic, but although its reported average specificity is good (99%-100%), its reported sensitivity is relatively low (51-80% at standard interpretation) (De La Rua-Domenech et al., 2006) resulting in this test being inclined to leave residual infection behind. Research in the Republic of Ireland highlighted the importance of this by attributing 15% of bTB episodes to residual infection (White et al., 2013). Chapter 6 of this thesis was dedicated to estimating the sensitivity and specificity of the SCITT test under the current Northern Irish circumstances.

Both a Hui-Walter model and an animal level model demonstrated comparable posterior median estimation for the sensitivity for SICCT test (92.0% and 89.79%, respectively). This was higher than the study previously conducted in Northern Ireland (Lahuerta-Marin et al., 2018a; reported sensitivity 40.5–57.7%). However, it has to be noted that the study conducted previously by Lahuerta-Marin et al. (2018a) focused on chronic bTB breakdown herds only suggesting that these are herds that are tested on short intervals for prolonged periods of time driving the sensitivity of the SICCT test down. Moreover, estimates for chronic bTB breakdown herds used gamma interferon test results for their analyses creating a bias towards herds that have already been censored through removal of SICCT test reactors during at least one previous recent herd SICCT test. Test characteristic estimates are often assumed to be independent of disease prevalence but this has been reported as not being the case. Differences in prevalence, alongside differences in distribution of animals in the various stages of disease, between studies can therefore act as a flag for differences in study population or study design (Brenner and Gefeller, 1997; Leeflang et al., 2007). The sensitivity estimates reported in Chapter 6 of this thesis were more in line with previously reported figures from England (Karolemeas et al.,

2012) than with the figures reported by studies in the Republic of Ireland (Clegg et al., 2011a). This is potentially due to the bTB prevalence in the cattle population in Northern Ireland showing more similarities with England than the Republic of Ireland (Abernethy et al., 2013). In addition to this, it has to be noted that the study described in this thesis was carried out in slaughtered cattle in Northern Ireland which may not be representative for the general cattle population.

The two models described in Chapter 6 of this thesis showed almost identical posterior medians for the specificity (≥ 99.98% from both models for SICCT test which is similar to previously reported figures (Goodchild et al., 2015; Nuñez-Garcia et al., 2017)). This shows that very few cattle that react positively to the SICCT test are not infected with bTB.

The importance of the estimation of test characteristics is that residual infection can be put into context of the diagnostic test performed. Indeed several studies have shown that residual infection plays a significant role in bTB infection being persistent. Berrian et al. (2012) and Wolfe et al. (2009) found that cattle moved from herds that had a history of bTB infection were more likely to being disclosed as a reactor compared to animals moved from herds without a recent bTB history. Further evidence of residual infection being left behind in a herd is based on animals that were classified as inconclusive to the SICCT test and therefore retained in the herd. These animals were at a higher risk of being disclosed as a reactor at a future test or being disclosed as positive at routine slaughter (Clegg et al, 2011b; Clegg et al., 2011c).

Residual infection also appears to be linked to the number of SICCT reactors disclosed during the bTB breakdown with breakdowns with multiple reactors having been found to being a risk factor for future bTB breakdown in several previous studies (Olea-Popelka et al., 2004; Abernethy et al., 2010; Wolfe et al., 2010; Murray et al., 2010; Doyle et al., 2014; Clegg et al., 2015). The study described in Chapter 5 in the current thesis specifically focussed on the issue of multiple SICCT reactor breakdown herds and future breakdowns in a Northern Ireland context and showed that the risk of a future bTB breakdown increases with the number of SICCT reactors disclosed during a breakdown,regardless of the confirmation status of the breakdown. These findings formed the scientific basis for a change in policy implemented in March 2018. Herds have their Officially Tuberculosis free status Withdrawn (OTW) when there is more than one reactor disclosed during the course of a breakdown, rather than more than five as previously. This means that any herd with more than one SICCT test reactor is restricted until it has completed at least two negative whole SICCT herd tests after removal of any reactors.

In addition to the SICCT test the Northern Ireland bTB programme uses the gamma interferon (IFNγ test) in a limited number of herd. This performance of this diagnostic test was not evaluated within the remit of this thesis.

Post-mortem inspection for visible bTB like lesions of all cattle routinely slaughtered takes place in Northern Ireland as an ancillary surveillance system (Pascual-Linaza et al., 2016). This is quite an important method of surveillance with 15-25% of positive bTB herds initially being detected by a positive abattoir case (Abernethy et al., 2013). The study described in Chapter 6 was the first study in Northern Ireland that estimated the test characteristics of routine post-mortem surveillance. This research reported a posterior median estimation for the sensitivity of post-mortem inspection to be 53.60% (Hui-Walter Model) and 52.88% (animal level model) respectively. Furthermore, the two models showed almost identical posterior median for the specificity (99.66% vs 99.63% respectively).

The study described in Chapter 6 also focused on applying a modelling method to the data in order to achieve a more precise test characteristic estimate (i.e. smaller credibility intervals). The Bayesian model specified at the animal level presented in this study offered the possibility of including animal level information such as risk factors for the estimation of test characteristics with the aim of making more precise estimates. This method was previously applied by Koop et al. (2013) in relation to mastitis in goats and was in Chapter 6 applied to bTB related data. Indeed the application of the Bayesian animal level model with risk factors (age at death, days from last test to slaughter, sex and last test reason) showed much narrower posterior credible intervals than the traditional Hui-Walter model, indicating more certainty for the estimates. This model is hoped in the future to be applied to populations that are possibly more representative of the general cattle population rather that the population used in Chapter 6 (i.e. all cattle slaughtered in 2015).

Chapter 2 of this thesis focussed specifically on the risk factors that affect the development of visible bTB lesions in SICCT test reactors at post-mortem examination, again linking back to improving post-mortem surveillance and increasing the understanding we have in relation to this. The awareness raised of these risk factors aid abattoir surveillance as they create more vigilance during the post mortem examination for animals in specific categories with the aim of improvement of the sensitivity of abattoir examination and knowledge of bTB lesion development.

Ownership/responsibility of bTB control

The farming industry is the main beneficiary of a successful bTB eradication programme. Nevertheless, in Northern Ireland and the rest of the British Isles, all aspects of the programme are currently controlled and directed by Government. Minimal involvement from farming industry is therefore currently in place in relation to governance (Robinson, 2014; More, 2009; More and Good, 2015). This has caused bTB being viewed as a 'Government problem' instead of being an infectious disease. This view has previously been stated to be exacerbated through the level of compensation paid for reactor animals which further shifts the responsibility to the Government instead of being in place to encourage farmer's behaviour in part taking in bTB control (TB Strategic Partnership Group, 2016). Moreover the lack of success of the current bTB control programmes has previously not only been quoted to result in a lack of trust in Government and science (Enticott, 2008), but also has created a sense of fatalism regarding the lack of control individual farmers feel they have in relation to bTB control on their own farm (Enticott et al., 2012; Enticott et al., 2015). This lack of progress has also let to 'fatigue' and renewed inspiration involving all stakeholders have been quoted by Robinson (2014) as one of the important steps in achieving bTB eradication. The importance of commitment and involvement of stakeholders such as the farming industry has been highlighted previously during eradication campaigns in other countries such as Australia (Radunz, 2006; More, 2008; More et al., 2015) and New Zealand (Tweddle and Livingstone, 1994).

Involvement of the farming industry in relation to bTB control can be at a variety of levels from policy making to farmer's taking ownership of bTB control on their own farm. In relation to the latter it is known that beliefs can play an important role in farmer behaviour and willingness to adopt new policies (Edward-Jones, 2006). In order to explore this in a Northern Ireland context, part of this dissertation aimed to assess the situation with regards to bTB related biosecurity measures implemented by farmers on their own farm and their attitudes towards bTB control (see Chapter 3 and 4). The two studies as described in Chapter 3 and 4 aimed, among having other objectives, to set a baseline level as to what farmers' current attitudes are towards bTB control and bTB related biosecurity in Northern Ireland. This part of the thesis encompassed the first research conducted into farmers' attitudes and baseline practices in relation to bTB control in Northern Ireland.

One of the main outcomes of the research conducted was that, in line with findings by Ward et al. (2010), there was a lack of adoption of bTB related biosecurity measures by farmers, especially in relation to badgers. These findings applied to both farms that had a recent history of bTB and farms that had been clear of bTB in recent years. Examples of this include: although most study farms used raised feed and water troughs, they were likely to have been installed for reasons other than increasing biosecurity (e.g. to prevent cattle defecating into troughs). Little was done (such as pre- or post-movement testing, maintaining a closed herd) to reduce the risk of bTB introduction by cattle purchase. Measures such as isolation, washing and disinfection were generally better adopted. Moreover, recent bTB breakdown farms were more inclined to purchase beef cattle (OR 4.60; 95% CI 1.61, 13.13), use contractors to spread slurry (OR 2.83; 95% CI 1.24, 6.49), feed meal on top of silage (OR 3.55; 95% CI 1.53, 8.23) and feed magnesium supplement (OR = 3.77; 95% CI 1.39, 10.17). These findings showed that adoption of biosecurity measures has much scope for improvement. In the years that followed since this study was conducted, Government has actively encouraged to increase farmers' participation in biosecurity in a range of ways including distributing leaflets and increasing awareness among private veterinary practitioners to make biosecurity advice to farmers as one of their important tasks.

Although these findings reaffirmed that there is an issue in relation to responsibility and ownership in relation to bTB control, the research outlined in Chapter 3 indicated that farmers agreed with the importance of bTB control in Northern Ireland and that they generally have a very positive attitude to bTB control measures regardless of the bTB history of their own herd. An additional important finding was that there was strong support for both badger culling and badger vaccination from the participating farmers, but that in general they would prefer a badger vaccination programme. This was an important finding for badger intervention programmes that have since been conducted and planned in Northern Ireland. Within the study there were few but some significant differences in relation to welcoming further advice or support for badger intervention suggesting that when farmers had actually experience an outbreak, it can change their beliefs in relation to certain issues (Enticott and Vanclay, 2011; Hernandez-Jover et al., 2012; Zingg and Siegrist, 2012). Furthermore the study pointed out that farmers regard the financial implications of bTB control measures often as a problem for themselves regardless of whether or not they had experienced a recent bTB breakdown in their herd highlighting again problems with attitudes in relation to the responsibility for the actual disease control. A similar view can be found in relation to control of bTB in wildlife. Enticott et al. (2012) who found in their research that the majority of farmers thought badger vaccination was a good thing to do, the vast majority of farmers do not think it is their responsibility to pay for badger vaccination which was in line with research described in Chapter 3 in this thesis. Since the research described in Chapter 3 was conducted further research has been conducted in relation to farmers' behaviour in relation to biosecurity (Lahuerta-Marin et al., 2018b).

Nature of the farming industry

Two of the main issues relating to the Northern Irish farming industry which facilitate disease transmission are the extensive farm fragmentation and substantial between herd animal movements, which both increase potential disease exposure between herds (TB Strategic Partnership Group, 2016).

Purchase of animals, i.e. not keeping a closed herd, has been identified as a potential source of infection in several studies (Clegg et al., 2008; Doyle et al., 2017). Approximately 6-7% of herd breakdowns were directly attributed to the purchase of an infected animal in these two studies. Indeed in the studies conducted within this thesis keeping a closed herd (i.e. no purchase of animals) is only common practice for a minority of farms in Northern Ireland. Chapter 4 of this thesis showed that

88.5% of study farmers stated that they had purchased cattle in the last 3 years. Similar figures were found in the study described in Chapter 5 with 86.6% of study farms purchasing cattle in the time period allocated in the study design.

Furthermore, the study described in Chapter 4 showed that recent bTB breakdown farms were more inclined to purchase beef cattle (OR 4.60; 95% CI 1.61-13.13) compared to farms that did not have a recent bTB breakdown. Moreover, purchase of cattle was identified as a significant risk factor ($p<0.001$) for future bTB breakdown in the study described in Chapter 5. This is in line with previously conducted studies (Johnston et al., 2005; Carrique-Mas et al., 2008; Ramírez-Villaescusa et al., 2010; Doyle et al., 2014; Doyle et al., 2017). Purchasing cattle however was not an identified risk factor in relation to confirmation status of reactors to the SICCT test (as described in Chapter 2 of this thesis).

Extensive farm fragmentation refers to small farms with extensive use of conacre land (i.e. land rented from other farm) and is a common farm management practice in Northern Ireland (Abernethy et al., 2006; Robinson, 2014; TB Strategic Partnership Group, 2016). Farm fragmentation increases the chances of disease transmission by increased probability of contact with neighbouring cattle or increased exposure to wildlife. Indeed, this problem was highlighted in the study outlined in Chapter 4 of this thesis which showed that there was a mean in excess of 8 boundaries per farm (range 1-33) with 66.8% of farm boundaries providing opportunities for nose-to-nose contact (based on allowance of a minimum gap between cattle < 3 m). Research in the Republic of Ireland highlighted the importance of this by attributing up to 20% of bTB episodes to contiguous spread (White et al., 2013).

Wildlife

The estimated badger population of Northern Ireland is 34,100 (95% CI 26,200-42,000) (Reid et al., 2008). It is a well-accepted fact that badgers act as a maintenance host on the British Isles with spill-back to cattle (More, 2009). Infection with *M. bovis* in badgers is widespread in Northern Ireland with the prevalence of bTB being estimated to be 15.3% (95% CI 13.1-17.5%) based on post mortem examination of badgers killed by road traffic accidents (Courcier et al., 2018). This estimate is similar to prevalence estimates found in other parts of the British Isles (8.2–27.2% in England and Wales (Goodchild et al., 2012); 10–14% in the Republic of Ireland (O'Boyle, 2002)). However these figures are likely to be under-estimates particular due to reliance on gross pathology with the risk of failing to detect non-visibly lesioned animals (Corner et al., 2011). In the Republic of Ireland it was found that areas with high bTB prevalence in cattle also have a high bTB prevalence in badgers and vice versa (Murphy et al., 2010). In addition to badgers known to being infected with bTB further consistent evidence of badgers playing a role in the persistence of bTB in cattle became evident during the East Offaly and the Four area trials in the Republic of Ireland. In these trials where removal of badgers were followed by a sustained significant fall in bTB prevalence in cattle in those areas (Griffin et al., 2005; Kelly et al., 2008; Olea-Popelka et al., 2009; Byrne et al., 2014). This was supported by findings from the Randomised Badger Culling Trial in England[1]. This has led to a national programme of badger culling being implemented since 2004 in the Republic of Ireland focusing especially on areas with chronic bTB problems in cattle herds (Sheridan, 2011). Moreover molecular typing data support a local epidemiological association between *M. bovis* in cattle and badgers with badgers and cattle tending to share the same *M. bovis* genotypes in the same areas (Olea-Popelka et al., 2005; Woodroffe et al., 2009; Allen et al, 2011). Further support the link between badgers and cattle was reported by Menzies et al. (2011) who found higher levels of badger activity in areas with high bTB incidence compared to areas with low bTB incidence.

The transmission of *Mycobacterium bovis* can potentially take place via direct (or close) contact between badgers and cattle (e.g. aerosol) or via indirect contact (e.g. urine, faeces). Intervention methods to prevent these routes of transmission are usually divided up in measures to be applied in farm buildings (in order to prevent badgers accessing cattle houses and feed storages) and measures applied at pastures (mainly focusing on prevention of indirect contact between badgers and

[1] Bovine TB: The Scientific Evidence. On line available on: http://webarchive.nationalarchives.gov.uk/20081108133322/http://www.defra.gov.uk/animalh/tb/isg/pdf/final_report.pdf

cattle) (Allen et al., 2011). Ward et al. (2005) stated that the physical exclusion of badgers from farm buildings is likely to be the simplest/most practical.

As bTB transmission routes likely vary in space and time (Allen et al., 2011), two studies were conducted in Northern Ireland in order to get a better understanding of badger related risk factors for bTB in the province. The first study conducted in this contexts dates back to 1999 (Denny and Wilesmith, 1999) and found an aetiological fraction of 40% for the association between the presence of badgers and bTB breakdowns in herds that could not be attributed to the purchase of infected cattle. The study described in Chapter 4 of this thesis was the second study conducted which had as one of its aim to research the association between badgers and bTB breakdown farms from a Northern Irish perspective. The latter study aimed, among having other objectives, to disentangle risk factors for bTB breakdown in relation wildlife. The study found that the odds of being a bTB breakdown farm were significantly increased by the presence of accessible badger setts and the observation of live badgers on the farm. It is known that although badger visits to farm building are infrequent (Sleeman et al., 2008; O'Mahoney, 2014) badgers are attracted by food and feed supplements and have been reported to visit feed stores and cattle houses (Phillips et al., 2003; Tolhurst et al., 2009; Ward et al., 2010; O'Mahoney, 2014). In line with this the study described in Chapter 4 showed that farmers with farms with a recent bTB breakdown were significantly more likely to feed magnesium supplement and meal on top of the silage highlighting a possible route of bTB transmission.

Further to these findings in relation to badger-related risk factors for bTB breakdown, valuable ecological information was obtained during the study described in Chapter 4 of this thesis including the high proportion of farms (~83%) that had badger setts, the accurateness of farmers identifying badger setts (positive predictive value 81.4%, 95% CI 74.9-86.5; negative predictive value 49.3%, 95% CI 37.7-60.9) and the high proportion badger setts on farms that were accessible to cattle (77.1%; 95% CI 70.4-82.7).

Wild deer can contract bTB and in England and Wales wild deer have been identified as a potential, localised source of bTB infection to cattle (Delahay et al., 2007; Ward et al., 2009). However in Northern Ireland the prevalence of *M. bovis* in wild deer is known to be low (~2%; Department of Agriculture and Rural Affairs, 2009). In the study outlined in Chapter 4 of this thesis no significant association was found between the presence of deer and the risk of being a bTB breakdown farm, consistent with previous findings by Denny and Wilesmith (1999).

Summary of findings and impacts

Summary of findings and impacts: Chapter 2

Risk factors for disclosing and laboratory confirmation of macroscopic lesions in cattle at slaughter were investigated as the visible lesion status and bTB status of an animal can affect the way in which bTB breakdowns are managed, since failure to detect visible lesions and recovery of *M. bovis* can lead to a less stringent follow-up. Focusing on reactor cattle 43.0% had visible lesions, whilst 45.3 % of those sampled were confirmed as bTB positive due to the presence of lesions or positive histopathology/mycobacterial culture (positive bTB status). Significant risk factors associated with VL status and bTB status found at multivariable analyses included age at death, breed, sex, test year, net increase in skin thickness at bovine tuberculin injection site, epidemiological status of skin test, total number of reactors at the disclosure test, mean herd size and prior response to the SICCT test.
These risk factors are likely related to the time since infection, the strength of the challenge of infection and the susceptibility of the animal. This study has contributed to a better awareness of risk factors for the development of bTB visible lesions and has been cited by several other studies since its publication (Broughan et al., 2016; Clegg et al., 2016; Clegg et al., 2017; Downs et al., 2016; Byrne et al., 2017; Byrne et al., 2018; Lahuerta-Marin et al., 2016; Nunez-Garcia et al., 2017)

Summary of findings and impacts: Chapter 3

Beliefs can play an important role in farmer behaviour and willingness to adopt new policies. In this study the views of farmers who had experienced a recent confirmed or multiple reactor bTB breakdowns were compared to those of farmers who had no recent reactors or restricted herd tests. All participating farmers found bTB control important and most were keen to learn more about bTB biosecurity measures and were in favour of the cattle-related bTB control measures (isolation of SICCT test inconclusive animals, use of the gamma-interferon test and pre-movement testing).

The majority of farmers would allow badger vaccination and culling on their own land with an overall preference for vaccination. Highest disagreement was shown in relation to willingness to pay for bTB control measures. There was agreement on most issues between different age groups of farmers. Farmers which experienced a recent breakdown however showed significantly more support for additional advice on bTB biosecurity measures and were also more in favour of allowing badger vaccination and culling on their land whilst showing less concern for public opposition. This study was the first research conducted in Northern Ireland examining farmers' attitudes to bTB control and it was therefore the first study conducted in relation to the social aspects surrounding bTB in the province. This study has been cited by several other studies both inside the British Isles and beyond (Spain, Brazil, Nigeria, Canada) since its publication (Moustakas and Evans, 2016; Ciaravino et al., 2017; Kraemer and Oppliger, 2017; Robinson, 2017a, b; Carneiro and Kaneene, 2018; Jajere et al., 2018; Sumner et al., 2018).

Summary of findings and impacts: Chapter 4

An observational case-control study was conducted in a high incidence area in 2010-2011. The aim was to identify risk factors for bTB breakdown relating to cattle and badgers and to assess the adoption of bTB related biosecurity measures on farms. Face-to-face questionnaires with farmers and surveys of badger setts and farm boundaries were conducted on 117 farms with (cases) and 75 farms without (controls) a recent bTB breakdown. Logistic regression at univariable and multivariable level disclosed significant risk factors associated with being a case herd, including having an accessible badger sett within the farm boundaries in a field grazed in the last year (odds ratio, OR, 4.14; 95% confidence interval, CI, 1.79, 9.55), observation of live badgers (OR 4.14; 95% CI 1.79, 9.55), purchase of beef cattle (OR 4.60; 95% CI 1.61, 13.13), use of contractors to spread slurry (OR 2.83; 95% CI 1.24, 6.49), feeding meal on top of silage (OR 3.55; 95% CI 1.53, 8.23) and feeding magnesium supplement (OR = 3.77; 95% CI 1.39, 10.17). The majority of setts within the farm boundary were stated to be accessible by cattle (77.1%; 95% CI 71.2, 83.0%) and 66.8% (95% CI 63.8, 69.7%) of farm boundaries provided opportunities for nose-to-nose contact between cattle. Adoption of bTB related biosecurity measures, especially with regards to purchasing cattle and badger-related measures, was lower than measures in relation to disinfection and washing.

Since its publication the study describe in Chapter 4 has been cited several times (Doyle et al., 2017; Kraemer and Oppliger, 2017; Robinson, P.A., 2017a, b; Lipiec et al., 2018; Richens et al., 2018).

Summary of findings and impacts: Chapter 5

This study utilising data extracted from the national animal health database in Northern Ireland used Kaplan Meier curves and univariable and multivariable Cox Proportional Hazard models to evaluate the effect of the number of SICCT reactors and bTB confirmation on the risk of future bTB herd incident. Based on multivariable analyses the risk of a future bTB herd incident was positively associated with the number of SICCT reactors identified during the incident period (Hazard Ratio=1.861 in incidents >5 SICCT reactors compared to incidents with only one SICCT reactor; P<0.001), but not with bTB confirmation. These findings suggest that the probability of residual bTB infection in a herd increases with an increasing number of SICCT reactors disclosed during a bTB herd incident. As a direct result of this study the policy imbedded in the Northern Ireland bTB programme was changed. Initially cattle herds were able to regain Official Tuberculosis Free (OTF) status after a bTB herd incident with 5 or less unconfirmed reactors by only needing one clear SICCT test. Currently two consecutively negative SICCT herd tests after disclosure of two or more reactors are required irrespective of bTB confirmation.

Summary of findings and impacts: Chapter 6

Latent class modelling is often used to estimate bTB test characteristics due to the absence of a gold standard. However, the reported sensitivity of especially the SICCT test has shown a lot of variation. Both the Hui-Walter latent class model under the Bayesian framework and the Bayesian model specified at the animal level, including various risk factors as predictors, were used to estimate the SICCT test and post-mortem test characteristics. Data were collected from all cattle slaughtered in abattoirs in Northern Ireland in 2015. Both models showed comparable posterior median estimation for sensitivity of the SICCT test (88.61% and 89.79%, respectively) and for post-mortem examination (53.65% and 52.88%, respectively). Both models showed almost identical posterior median estimates for the specificity (99.99% from both models for SICCT test and 99.66% vs 99.63% for post-mortem examination). However, the animal level model showed much narrower posterior

95% credible intervals, suggesting more certainty for the estimates. This study was carried out in slaughtered cattle which may not be representative for the general cattle population, but it is arguably more representative than other studies that have usually used data censored by short-interval serial SICCT tested bTB breakdowns herds.

Recommended future research

In order to achieve eradication of bTB, science and policy need to have a very clear link (More and Good, 2006). Therefore research in relation to bTB needs to be driven by focussed questions that once answered lead to appropriate action by policy. This approach should underpin the main problems that currently stand in the way of achieving bTB control and ultimately eradication.

This thesis has underlined some of the main problems that the Northern Ireland cattle industry currently faces in relation to bTB. Although a large platform of research has been conducted in the past and is still being conducted currently in Northern Ireland and beyond, there are still areas that need further attention. Some research however, such as the link with social science (for example in relation to farmers' attitudes and behaviours) has only recently been established and needs further and more detailed attention. Other issues highlighted in this thesis such as problems in relation to farming practices are however not only depended on farmers' beliefs and behaviours but are also deeply imbedded into the farming culture and its industry in Northern Ireland (Robinson, 2014) and therefore will involve a difficult and lengthy process before a change can be noticed. However, further research not only directly related to the social aspects (for example drivers and motivations of farmer's behaviour) of the bTB persistence in Northern Ireland but also directly science related research (for example in relation to improving surveillance) should help to ultimately achieve the goal of bTB eradication.

Conclusion

In conclusion, bTB in cattle is a complex disease with important impact for economic, zoonotic and animal welfare reasons. A comprehensive bTB programme exists in Northern Ireland but many challenges are still to be faced in relation to for example the nature of the farming industry and diagnostics applied. Although this thesis has aimed to address some of these difficulties, further data collection and analysis are very relevant on an ongoing basis to tailor the bTB programme and its implication to the best it can be. Further control and ultimately eradication of bTB will strongly rely on a well-constructed science-informed input and guidance.

References

Abernethy, D.A., Denny, G.O., Menzies, F.D., McGuckian, P., Honhold, N., Roberts, A.R. (2006): The Northern Ireland programme for the control and eradication of *Mycobacterium bovis.* Veterinary Microbiology 112, 231-237

Abernethy, D.A., Graham, D., Skuce, R., Gordon, A., Menzies, F., Robinson, P., Harwood, R., Clarke, N. (2010): Shifting the TB-control paradigm: an epidemiological critique of the bovine tuberculosis eradication scheme. Society for Veterinary Epidemiology and Preventive Medicine. Proceedings, Nantes, France, 24-26 March, 2010, 166-173

Abernethy, D.A., Upton, P., Higgins, I.M., McGrath, G., Goodchild, A.V., Rolfe, S.J., Broughan, J.M., Downs, S.H., Clifton-Hadley, R., Menzies, F.D., de la Rua-Domenech, R., Blissit, M.J., Duignan, A., More, S.J. (2013): Bovine tuberculosis trends in the UK and Republic of Ireland, 1995-2010. Veterinary Record 172, 312. doi: 10.1136/vr.100969

Allen, A.R., Skuce, R.A., McDowell, S.W.J. (2011): Bovine TB (TB): a review of badger-to-cattle transmission. On line available on: https://www.daera-ni.gov.uk/sites/default/files/afbi-literature-review-tb-review-badger-to-cattle-transmission.pdf (Accessed on 09/04/2018)

Berrian, A.M., O'Keeffe, J.J., White, P., Norris, J., Litt, J., More, S.J., Olea-Popelka, F.J. (2012): Risk of bovine tuberculosis for cattle sold out from herds during 2005 in Ireland. Veterinary Record 170, 620

Brenner, H., Gefeller, O. (1997): Variation of sensitivity, specificity, likelihood ratios and predictive values with disease prevalence. Statistics in Medicine 16, 981-991

Broughan, J.M., Judge, J., Ely, E., Delahay, R.J., Wilson, G., Clifton-Hadley, R.S., Goodchild, A.V., Bishop, H., Parry, J.E., Downs, S.H. (2016): A review of risk factors for bovine tuberculosis infection in cattle in the UK and Ireland. Epidemiology and Infection 1 (14), 1-28

Byrne, A.W., White, P., McGrath, G., O'Keeffe, J., Martin, S.W. (2014): Risk of tuberculosis cattle herd breakdowns in Ireland: effects of badger culling effort, density and historic large-scale interventions. Veterinary Research 45,109

Byrne, A.W., Graham, J., Brown, C., Donaghy, A., Guelbenzu-Gonzalo, M., McNair, J., Skuce, R.A., Allen, A., McDowell, S.W. (2017): Bovine tuberculosis visible lesions in cattle culled during herd breakdowns: the effects of individual characteristics, trade movement and co-infection. BMC Veterinary Research 13:400

Byrne, A.W., Graham, J., Brown, C., Donaghy, A., Guelbenzu-Gonzalo, M., McNair, J., Skuce, R.A., Allen, A., McDowell, S.W. (2018): Modelling the variation in skin-test tuberculin reactions, post-mortem lesion counts and case pathology in tuberculosis-exposed cattle: Effects of animal characteristics, histories and co-infections. Transboundary and Emerging Diseases 65(3), 844-858

Carneiro, P., Kaneene, J.B. (2018): Bovine tuberculosis control and eradication in Brazil: Lessons learned from the US and Australia. Food Control 93, 61-69

Carrique-Mas, J.J., Medley, G.F., Green, L.E. (2008). Risks for bovine tuberculosis in British cattle farms restocked after the foot and mouth disease epidemic of 2001. Preventive Veterinary Medicine 84, 85-93

Ciaravino, G., Ibarra, P., Casal, E., Lopez, S., Espluga, J., Casal, J., Napp, S., Allepuz, A. (2017): Farmer and veterinarian attitudes towards the bovine tuberculosis programme in Spain: what is going on in the field? Frontiers in Veterinary Science 4, 202, doi: 10.3389/fvets.2017.00202

Clegg, T.A., More, S.J., Higgins, I.M., Good, M., Blake, M., Williams, D.H. (2008): Potential infection control benefit for Ireland from pre-movement testing of cattle for tuberculosis. Preventive Veterinary Medicine 84 (1-2), 94-111

Clegg, T.A., Duignan, A., Whelan, C., Gormley, E., Good, M., Clarke, J., Toft, N., More, S.J. (2011a). Using latent class analyses to estimate the test characteristics of the γ-interferon test, the single intradermal comparative tuberculin test and a multiplex immunoassay under Irish conditions. Veterinary Microbiology 151, 86-76

Clegg, T.A., Good, M., Duignan, M., Doyle, R., Blake, M. and More, S.J. (2011b): Longer-term risk of *Mycobacterium bovis* in Irish cattle following an inconclusive diagnosis to the single intradermal comparative tuberculin test. Preventive Veterinary Medicine 100, 147-154

Clegg, T.A., Good, M., Duignan, M., Doyle, R., More, S.J. (2011c): Shorter-term risk of *Mycobacterium bovis* in Irish cattle following an inconclusive diagnosis to the single intradermal comparative tuberculin test. Preventive Veterinary Medicine 102, 255-264

Clegg, T.A., Good, M., More, S.J. (2015): Future risk of bovine tuberculosis recurrence among higher risk herds in Ireland. Preventive Veterinary Medicine 118, 71-79

Clegg, T.A., Good, M. and More, S.J. (2016): Risk factors for cattle presenting with a confirmed bTB lesion at slaughter, from herds with no evidence of within-herd transmission. Preventive Veterinary Medicine 126, 111-120

Clegg, T.A., Good, M., Doyle, M., Duignan, A., Gormley, E. (2017): The performance of the interferon-gamma assay when used as a diagnostic or quality assurance test in *Mycobacterium bovis* infected herds. Preventive Veterinary Medicine 140, 116-121

Corner, L.A., Murphy, D., Gormley, E. (2011): *Mycobacterium bovis* infection in the Eurasian badger (*Meles meles*): the disease, pathogenesis, epidemiology and control. Journal of Comparative Pathology 144, 1–24

Courcier, E.A., Menzies, F.D., Strain, S.A.J., Skuce, R.A., Robinson, P.A., Patterson, T., McBride, K.R., McCormick, C.M., Walton, E., McDowell, S.W.J., Abernethy D.A. (2018): *Mycobacterium bovis* surveillance in Eurasian badgers (*Meles meles*) killed by vehicles in Northern Ireland between 1998 and 2011. Veterinary Record 182(9), 259. doi: 10.1136/vr.103934

Delahay, R. J., Smith, G. C., Barlow, A. M., Walker, N., Harris, A., Clifton-Hadley, R. S., Cheeseman, C. L. (2007): Bovine tuberculosis infection in wild mammals in the South-West region of England: A survey of prevalence and a semi-quantitative assessment of the relative risks to cattle. Veterinary Journal 173(2), 287–301

De la Rua-Domenech, R., Goodchild, A.T., Vordermeier, H.M., Hewinson, R.G., Christiansen, K.H., Clifton-Hadley, R.S. (2006): Ante mortem diagnosis of tuberculosis in cattle: a review of the tuberculin tests, gamma-interferon assay and other ancillary diagnostic techniques. Research in Veterinary Science 81,190–210. doi: 10.1016/j.rvsc.2005.11.005

Department of Agriculture and Rural Affairs (2009): Wild deer TB surveillance 2008/2009. On line available at: https://www.daera-ni.gov.uk/articles/wild-deer-tb-surveillance-200809 (Accessed 05 April 2018)

Department of Agriculture, Environment and Rural Affairs (2017): Statistical review of Northern Ireland Agriculture 2016. On line available at: https://www.daera-ni.gov.uk/sites/default/files/publications/daera/Stats%20Review%202017%20final.pdf (Accessed 31/08/2018)

Denny, G.O., Wilesmith, J.W. (1999): Bovine tuberculosis in Northern Ireland: a case-control study of herd risk factors. Veterinary Record 144, 305-310

Downs, S.H., Broughan, J.M., Goodchild, A.V., Upton, P.A., Durr, P.A. (2016): Responses to diagnostic tests for bovine tuberculosis in dairy and non-dairy cattle naturally exposed to *Mycobacterium bovis* in Great Britain. Veterinary Journal 216, 8-17

Doyle, L.P., Gordon, A.W., Abernethy, D.A., Stevens, K. (2014): Bovine tuberculosis in Northern Ireland: risk factors associated with time from post-outbreak test to subsequent herd breakdown. Preventive Veterinary Medicine 116 (1-2), 47-55

Doyle, L.P., Courcier, E.A., Gordon, A.W., O'Hagan, M.J.H., Stegeman, J.A., Menzies, F.D. (2017): Bovine tuberculosis in Northern Ireland: quantification of the population disease level effect from cattle leaving herds detected as a source of infection. Epidemiology and Infection 145(16), 3505-3515

Edward-Jones, G. (2006): Modelling farmer decision making: concept, progress and challenges. Animal Science 82, 783-790

Enticott, G. (2008): The ecological paradox: Social and natural consequences of the geographies of animal health promotion. Transactions of the Institute of British Geographers 33, 433-446

Enticott, G., Vanclay, F. (2011): Scripts, animal health and biosecurity: The moral accountability of farmers' talk about animal health risks. Health Risk and Society 13, 293-309

Enticott, G., Maye, D., Ilbery, B., Fisher, R., Kirwan, J. (2012): Farmers' confidence in vaccinating badgers against bovine tuberculosis. Veterinary Record 170, 204

Enticott, G., Maye, D., Carmody, P., Naylor, R., Ward, K., Hinchliffe, S., Wint, W., Alexander, N., Elgin, R., Ashton, A., Upton, P., Nicholson, R., Goodchild, T., Brunton, L., Broughan, J. (2015) Farming on the edge: farmer attitudes to bovine tuberculosis in newly endemic areas. Veterinary Record, 177 (17). 439

European Commission (2014): Factsheet on 2014-2020 Rural Development Programme for Northern Ireland, Brussels, European Commission. Online available at: https://ec.europa.eu/agriculture/sites/agriculture/files/rural-development-2014-2020/country-files/uk/factsheet-northern-ireland_en.pdf (Accessed 25/01/2018)

Goodchild, A.V., Watkins, G.H., Sayers, A.R., Jones, J.R., Clifton-Hadley, R.S. (2012): Geographical association between the genotype of bovine tuberculosis found in dead badgers and in cattle herds. Veterinary Record 170, 259

Goodchild, A.V., Downs, S.H., Upton, P., Wood, J.L.N., de la Rua-Domenech, R. (2015): Specificity of the comparative skin test for bovine tuberculosis in Great Britain. Veterinary Record 177, 258-267

Griffin, J.M., Williams, D.H., Kelly, G.E., Clegg, T.A., O'Boyle, I., Collins, J.D., More, S.J. (2005): The impact of badger removal on the control of tuberculosis in cattle herds in Ireland. Preventive Veterinary Medicine 67, 237-266

Hernandez-Jover, M., Taylor, M., Holyoake, P., Dhand, N., 2012. Pig producers' perceptions of the Influenza Pandemic H1N1/09 outbreak and its effect on their biosecurity practices in Australia. Preventive Veterinary Medicine 106, 284-294

Jajere, S.M., Atsanda, N.N., Bitrus, A.A., Hamisu, T.M., Goni, M.D. (2018): A retrospective study of bovine tuberculosis at the municipal abattoir of Bauchi state, Northeastern Nigeria. Veterinary World 11(5), 598-605

Johnston, W.T., Gettinby, G., Cox, D.R., Bourne, F.J., Clifton-Hadley, R., le Fevre, A.M., McInerney, J.P., Mitchell, A., Morrison, W.I., Woodroffe, R. (2005): Herd-level risk factors associated with tuberculosis breakdowns among cattle in England before the 2001 foot-and-mouth disease epidemic. Biology letters 1(1), 53-56

Karolemeas, K., de la Rua-Domenech, R., Cooper, R., Goodchild, A.V., Clifton-Hadley, R.S., Conlan, A.J.K., Mitchell, A.P., Hewinson, R.G., Donnelly, C.A., Wood, J.L.N., McKinley, T.J. (2012): Estimation of relative sensitivity of the comparative tuberculin skin test in tuberculous cattle herds subjected to depopulation. *PLoS ONE* 7, e43217

Kelly, G.E., Condon, J., More, S.J., Dolan, L., Higgins, I., Eves, J. (2008): A long term observational study of the impact of badger removal on herd restrictions due to bovine TB in the Irish midlands during 1989–2004. Epidemiology and Infection 136, 1362–1373

Koop, G., Collar, C.A., Toft, N., Nielen, M., van Werven, T., Bacon, D., Gardner, I.A. (2013): Risk factors for subclinical intramammary infection in dairy goats in two longitudinal field studies evaluated by Bayesian logistic regression. Preventive Veterinary Medicine 108, 304-312

Kraemer, J., Oppliger, A. (2017): What is known about zoonotic bacteria in cattle and pig farms, and what should be done to prevent occupational infections. In book: Exposure to Microbiological Agents in Indoor and Occupational Environments, doi: 10.1007/978-3-319-61688-9_18

Lahuerta-Marin, A., McNair, J., Skuce, R. A., McBride, S., Allen, M., Strain, S.A.J., Menzies, F.D., McDowell, S.J.W., Byrne, A.W. (2016): Risk factors for failure to detect bovine tuberculosis in cattle from infected herds across Northern Ireland (2004-2010). Research in Veterinary Science 107, doi 10.2016/j.rvsc.2016.06.014

Lahuerta-Marin, A., Milne, M.G., McNair, J., Skuce, R.A., McBride, S.H., Menzies, F.D., McDowell, S.J.W., Byrne, A.W., Handel, I.G., de C. Bronsvoort, B.M. (2018a): Bayesian latent class estimation of sensitivity and specificity parameters of diagnostic tests for bovine tuberculosis in chronically infected herds in Northern Ireland. Veterinary Journal 238, 15-21

Lahuerta-Marin, A., Brennan, M.L., Finney, G., O'Hagan, M.J.H., Jack, C.G. (2018b): Key actors in driving behavioural change in relation to on-farm biosecurity; A Northern Ireland perspective. Irish Veterinary Journal 71:14. doi: 10.1186/s13620-018-0125-1

Leeflang, M. M., Bossuyt, P., Irwig, L. (2007): Sensitivity and specificity do vary with disease prevalence: implications for systematic reviews of diagnostic test accuracy. In: Evidence-based health care for all. Abstracts of the 15th Cochrane Colloquium; 2007 23-27 Oct; Sao Paulo, Brazil

Lipiec, M., Nowakowski, K., Radulski, L., Iwaniak, W., Wazna, A. (2018): Badgers as a potential source of bovine tuberculosis – first studies in Poland. Annals of Agriculture and Environmental Medicine, doi:10.26444/aaem/80984

Menzies, F.D., Abernethy, D.A., Stringer, L.A., Jordan, C. (2011): A comparison of badger activity in two areas of high and low bovine tuberculosis incidence of Northern Ireland. Veterinary Microbiology 151, 112-119

More, S.J., Good, M. (2006): The tuberculosis eradication programme in Ireland: a review of scientific and policy advances since 1988. Veterinary Microbiology 112, 239-251

More, S.J. (2008): A case for increased private sector involvement in Ireland's national animal health services. Irish Veterinary Journal 61, 70-78

More, S.J. (2009): What is needed to eradicate bovine tuberculosis successfully: an Irish perspective. Veterinary Journal 180(3), 275-278

More, S.J., Good, M. (2015): Understanding and managing bTB risk: Perspectives from Ireland. Veterinary Microbiology 176(3-4), 209-218

More, S.J., Radunz, B., Glanville, R.J. (2015): Lessons learned during the successful eradication of bovine tuberculosis from Australia. Veterinary Record 177, 224-232: doi: 10.1136/vr.103163

Moustakas, A., Evans, M.R. (2016): A big-data spatial, temporal and network analysis of bovine tuberculosis between wildlife (badgers) and cattle. Stochastic Environmental Research and Risk Assessment 31(2), 315-328

Murphy, D., Gormley, E., Costello, E., O'Meara, D., Corner, L.A.L. (2010): The prevalence and distribution of *Mycobacterium bovis* infection in European badgers (*Meles meles*) as determined by enhanced post- mortem examination and bacteriological culture. Research in Veterinary Science 88, 1–5

Murray, D., Clegg, T.A., More, S.J. (2012): Evaluation of single reactor bovine tuberculosis incidents based on analysis of reactors slaughtered at an Irish Export meat plant. Veterinary Record 170, 516-521

Nuñez-Garcia, J., Downs, S.H., Parry, J.E., Abernethy, D.A., Broughan, J.M., Cameron, A.R., Cook, A.J., De La Rua-domenech, R., Goodchild, A.V., Gunn, J., More, S.J., Rhodes, S., Rolfe, S., Sharp, M., Upton, P.A., Vordermeier, H.M., Watson, E., Welsh, M., Whelan, A.O., Woolliams, J.A., Clifton-hadley, R.S. and Greiner, M. (2017): Meta-analyses of the sensitivity and specificity of ante-mortem and post-mortem diagnostic tests for bovine tuberculosis in the UK and Ireland. Preventive Veterinary Medicine 153, 94-107

O'Boyle, I. (2002): Tuberculosis investigation unit, selected papers from 1997 to 2002: University College Dublin

Olea-Popelka, F.J., White, P.W., Collins, J.D., O'Keeffe, J,J, Kelton, D.F., Martin, S.W. (2004): Breakdown severity during a bovine tuberculosis episode as a predictor of future herd incidents in Ireland. Preventive Veterinary Medicine 63, 163-172

Olea-Popelka, F.J., Flynn, O., Costello, E. McGrath, G., Collins, J.D., O'Keefe, J., Kelton, D.F., Berke, O., Martin, S.W. (2005): Spatial relationship between *Mycobacterium bovis* strains in cattle and badgers in four areas in Ireland. Preventive Veterinary Medicine 71, 57-70

Olea-Popelka, F.J., Fitzgerald, P., White, P., McGrath, G., Collins, J.D., O'Keeffe, J., Kelton, D.F., Berke, O., More, S.J., Martin, S.W. (2009): Targeted badger removal and the subsequent risk of bovine tuberculosis in cattle herds in county Laois, Ireland. Preventive Veterinary Medicine 88, 178–184

O'Mahoney, D.T. (2014): Badger-cattle interactions in the rural environment: implications for bovine tuberculosis transmission. On line available at: https://www.daera-ni.gov.uk/articles/interactions-between-badgers-and-cattle-rural-environment (Accessed 05/04/2018)

Pascual-Linaza, A.V., Gordon, A.W., Stringer, L.A., Menzies, F.D. (2016): Efficiency of slaughterhouse surveillance for the detection of bovine tuberculosis in cattle in Northern Ireland. Epidemiology and Infection 145(5), 995-1005

Phillips, C.J.C., Foster, C.R.W., Morris, P.A., Teverson, R. (2003): The transmission of *Mycobacterium bovis* infection to cattle. Research in Veterinary Science 74, 1-5

Radunz, B. (2006): Surveillance and risk management during the latter stages of eradication: Experiences from Australia. Veterinary Microbiology 112, 283-290

Ramírez-Villaescusa, A.M., Medley, G.F., Mason, S., Green, L.E. (2010): Risk factors for herd breakdown with bovine tuberculosis in 148 cattle herds in the south west of England. Preventative Veterinary Medicine 95, 224-230

Reid, N., Etherington, T.R., Wilson, G.J., Montgomery, W.I., MacDonald, R.A. (2012): Monitoring and population estimation of the European badger *Meles meles* in Northern Ireland. Wildlife Biology 18, 46–57

Richens, I.F., Houdmont, J., Wapenaar, W., Shortall, O., Kaler, J., O'Connor, H., Brennan, M.L. (2018): Application of multiple behaviour change models to identify determinants of farmers' biosecurity attitudes and behaviours. Preventive Veterinary Medicine 155, 61-74

Robinson, P.A. (2014): A political ecology of bovine tuberculosis eradication in Northern Ireland, Durham thesis, Durham University. Available at Durham Thesis online: http://etheses.dur.ac.uk/10796/1/Philip_Robinson_Final_PhD_thesis.pdf (Accessed 25/01/2018)

Robinson, P.A. (2017a): Framing bovine tuberculosis, Contextualising statutory disease control with everyday farming lives. Geographical Journal 183 (3), 285-294

Robinson, P.A. (2017b): Farmers and bovine tuberculosis: 'A political ecology of health' approach to the circulation knowledge(s) about animal disease control. Journal of Rural Studies 55, 168-180

Sheridan, M. (2011): Progress in tuberculosis eradication in Ireland. Veterinary Microbiology 151,160–169

Sleeman, D.P, Davenport, J., Fitzgerald, A. (2008): Incidence of visits by badgers to farmyards in Ireland in winter. Veterinary Record 163, 734

Sumner, C.L., von Keyserlingk, M.A.G., Weary, D.M. (2018): Perspectives of farmers and veterinarians concerning dairy cattle welfare. Animal Frontiers 8(1), 8-13. doi: 10.1093/af/vfx006

TB Strategic Partnership Group (2016): Bovine Tuberculosis Eradication Strategy for Northern Ireland. Available on line at: https://www.daera-ni.gov.uk/sites/default/files/publications/daera/bovine-tuberculosis-eradication-strategy.pdf (Accessed 25/01/2018)

Tolhurst, B.A., Delahay, R.J.., Walker, A.I., Ward, A.I., Roper, T.J. (2009): Behaviour of badgers (*Meles meles*) in farm buildings: Opportunities for the transmission of *Mycobacterium bovis* to cattle. Applied Animal Behaviour Science 117, 103-113.

Tweddle, N.E., Livingstone, P. (1994): Bovine tuberculosis control and eradication programs in Australia and New Zealand. Veterinary Microbiology 40 (1-2), 22-39

Ward, A.I., Smith, G.C., Etherington, T.R., Delahay, R.J. (2009): Estimating the risk of cattle exposure to tuberculosis posed by wild deer relative to badgers in England and Wales. Journal of Wildlife Diseases 45(4), 1104–1120

Ward, A.I., Judge, J., Delahay, R.J. (2010): Farm husbandry and badger behaviour: Opportunities to manage badger to cattle transmission of *Mycobacterium bovis*? Preventive Veterinary Medicine 93, 2-10

White, P., Martin, S.W., De Jong, M.C.M., O'Keeffe, J.J., More, S.J., Frankena, K. (2013): The importance of "neighbourhood" in the persistence of bovine tuberculosis in Irish cattle herds. Preventive Veterinary Medicine 110, 346-355

Wolfe, D.M., Berke, O., More, S.J., Kelton, D.F., White, P.W., O'Keeffe, J.J., Martin, S.W. (2009): The risk of a positive test for bovine tuberculosis in cattle purchased from herds with and without a recent history of bovine tuberculosis in Northern Ireland. Preventive Veterinary Medicine 92, 99-105

Wolfe, D.M., Berke, O., Kelton, D.F., White, P.W., More, S.J., O'Keeffe, J., Martin, S.W. (2010): From explanation to prediction: A model for recurrent bovine tuberculosis in Irish cattle herds. Preventive Veterinary Medicine 94, 170-177

Woodroffe, R., Donnelly, C.A., Cox, D.R., Gilks, P., Jenkins, H.E., Johnston, W.T., Le Fevre, A.M., Bourne, F.J., Cheeseman, C.L., Clifton-Hadley, R.S., Gettinby, G., Hewinson, R.G., McInerney, J.P., Mitchell, A.P., Morrison, W.I., Watkins, G.H. (2009): Bovine TB in cattle and badgers in localised culling areas. Journal of Wildlife Diseases 45, 128-143

Zingg, A., Siegrist, M. (2012): Lay people's and experts' risk perception and acceptance of vaccination and culling strategies to fight animal epidemics. Journal of Risk Research 15, 53-66

Chapter 8

Summary

Summary

Bovine tuberculosis (bTB), caused by *Mycobacterium bovis,* has a global distribution and is one of the most important infectious diseases affecting the cattle industry in Northern Ireland. Alongside implications for animal and human health, it has significant economic consequences. Although infection primarily affects cattle, other mammals including humans, badgers, deer and many domestic animals can be infected as well. In cattle, bTB is mainly a respiratory disease but clinical signs are rare nowadays due to a compulsory eradication programme being in place since 1959 in Northern Ireland. However, even after decades of an eradication programme being applied based on testing and compulsory removal of positive animals, several obstacles are still causing bTB to persist in the cattle population. This persistence of bTB infection is due to a variety of reasons including residual infection within the herd, recurrent infection of herds and persistence of infection in the locality.

This thesis has addressed some of the problems relating to this persistence of infection focussing on surveillance, biosecurity and farmers' attitudes in relation to bTB control. The main issues considered included:

- the imperfect sensitivity of the currently available diagnostic tests leading to infected animals being left behind in a herd
- the little sense of ownership by farmers in relation to bTB control
- the nature of the farming industry in Northern Ireland including high herd and cattle density, small farm unit size and extensive use of outlying, rented pasture, significant between-herd movement and winter housing of cattle
- the presence of a wildlife reservoir (European badger, *Meles meles*)

Surveillance for bTB in Northern Ireland is mainly based on a combination of the single intradermal comparative cervical tuberculin (SICCT) test and routine post-mortem examination. Knowledge of diagnostic test characteristics is important in order to estimate how many infected animals are potentially left behind in a herd without being disclosed. The method applied to obtain these test characteristics estimates in this thesis included a Hui-Walter latent class model and a Bayesian model specified at the animal level including risk factors. The results showed that both tests were found to have a near perfect specificity but the reported sensitivity

was not for either test resulting in these tests potentially leaving infected animals behind. Furthermore, comparison of these figures with results reported in previous studies also highlighted that although test characteristic estimates are often assumed to be independent of disease prevalence this appears not to be the case. Differences in prevalence, alongside differences in distribution of animals in the various stages of disease, between studies can therefore act as a flag for differences in study population or study design.

Residual infection was found to be linked to the number of SICCT test reactors disclosed during the bTB breakdown with breakdowns with multiple reactors having been found to being a risk factor for future bTB breakdown regardless of the confirmation status of the breakdown. These findings formed the scientific basis for a change in policy implemented in March 2018 in Northern Ireland. Now, herds with multiple reactors are subjected to a stricter follow-up testing regime than before.

Focusing further on improvement of surveillance, and specifically on post-mortem inspection, Chapter 2 of this thesis evaluated potential risk factors that affect the development of visible bTB lesions in SICCT test reactors. The risk factors disclosed included age at death, breed, sex, test year, net increase in skin thickness at bovine tuberculin injection site, epidemiological status of skin test, total number of reactors at the disclosure test, mean herd size and prior response to the skin test. Knowledge of these risk factors aid abattoir surveillance as they create more vigilance during the post mortem examination for animals in specific categories.

Social aspects of bTB control are also considered as a challenge. In Northern Ireland and the rest of the British Isles, all aspects of the bTB eradication programme are currently controlled and directed by Government. This lack of direct involvement of the farming industry in relation to governance has lead to the studies into farmers' attitudes and behaviour being conducted as described in Chapters 3 and 4 of this thesis. These studies set a baseline level as to what farmers' current attitudes are towards bTB control and bTB related biosecurity in Northern Ireland. It was found that there was a lack of adoption of bTB related biosecurity measures by farmers, especially in relation to badgers. These findings applied to both farms that had a recent history of bTB and farms that had been clear of bTB in recent years. Furthermore, little was done by farmers to reduce the risk of bTB introduction by cattle purchase. These findings showed that adoption of biosecurity measures has much scope for improvement. In the years that followed since this study was conducted, Government has actively encouraged to increase farmers' participation in biosecurity. An additional important finding was that there was strong support for both badger culling and badger vaccination from the participating farmers, but that in general they would prefer a badger vaccination programme. This was an important finding for badger intervention programmes that have since been conducted and planned in Northern Ireland. Furthermore the financial implications of bTB control measures for farmers were disclosed as being a stumbling block.

It is a well-accepted fact that badgers act as a maintenance host for bTB on the British Isles. The case-control study described in Chapter 4 researched the association between badgers and farm management practices and bTB breakdown on farms from a Northern Irish perspective. The risk factors for bTB breakdown disclosed included the presence of accessible badger setts, the observation of live badgers on the farm and feeding magnesium supplement and meal on top of the silage. Furthermore a high proportion of farms was found to have badger setts that were accessible to cattle and farmers were found to be mostly accurate in identifying badger setts. The potential for nose-to-nose contact and therefore bTB transmission between cattle of neighbouring herds was also found to be cause for concern.

In conclusion, bTB in cattle is a complex disease with many challenges still to be faced in relation to for example the nature of the farming industry and diagnostics applied. However, this thesis has aimed to address some of these difficulties, which are hoped to contribute along with further data collection and analysis, to further control and ultimately eradicate bTB in Northern Ireland.

Chapter 9

Samenvatting

Samenvatting

Runder tuberculose, veroorzaakt door *Mycobacterium bovis,* heeft een wereldwijde distributie en is een van de belangerijkste ziektes voor the rundveehouderij in Noord Ireland. De ziekte heeft implicaties voor de gezondheid voor mens en dier en heeft belangerijke economische consequenties. De bacterie tast met name rundvee aan, maar mensen en andere zoogdieren zoals dassen, herten en gedomesticeerde dieren kunnen ook geinfecteerd raken. Runder tuberculose is met name een ziekte van het respiratie apparaat, maar klinische symptomen komen nu zelden voor vanwege het feit dat er sinds 1959 in Noord Ierland een eradicatie programma is. Er zijn echter obstakels die er voor zorgen dat runder tuberculosis nog steeds voorkomt in de rundvee populatie zelfs nadat er al tientallen jaren een eradicatie programma wordt toegepast gebaseerd op testen en verplicht slachten van test positieve dieren. Het feit dat runder tuberculose nog steeds voorkomt heeft verschillende redenen zoals infectie die in de kudde is achter blijft nadat de dieren zijn getest, herintroductie van infectie in de kudde en het achterblijven van infectie in de omgeving.

Dit proefschrift heeft enkele van deze problemen geadresseerd met speciale focus op bewaking, biosecurity en de houdingen van veehouders ten opzichte van runder tuberculose. De belangerijkste kwesties die zijn overwogen zijn:

- het feit dat de sensitiviteit van de diagnostische testen die beschikbaar zijn niet perfect is
- de minimale verantwoordelijkheid van veehouders voor de controle van runder tuberculose
- de structuur van de rundveehouderij in Noord Ierland gebaseerd op veel rundvee in kleine kuddes, kleine boerderijen, land fragmentatie, veel kopen en verkopen van rundvee en het overwinteren op stal
- het feit dat wild (das, *Meles meles*) geinfecteerd kan raken en infectie kan verspreiden

De bewaking voor runder tuberculose in Noord Ierland is met name gebaseerd op een combinatie van de single intradermal comparative cervical tuberculin (SICCT) test en routine onderzoek na het slachten.Kennis ten opzichte van de sensitiviteit and specificiteit van diagnostische testen is belangerijk omdat het een schatting kan geven over hoe veel geinfecteerde dieren in een kudde achter gelaten worden nadat de kudde is getest. De methodes die in dit proefschrift werden toegepast om deze kennis te verkrijgen waren een Hui-Walter latent class model en een Bayesian model op het niveau van het individuele dier met toegevoegde risico factoren. Dit proefschrift beschrijft dat beide diagnostische methodes een nagenoeg perfecte specificiteit hebben maar dat the sensitiviteit lager is. Dit is een van de oorzaken dat geinfecteerde dieren in de kudde achter blijven. Na vergelijking van de resultaten met eerdere studies werd het ook duidelijk dat alhoewel het vaak wordt aangenomen dat sensitiviteit en specificiteit onafhankelijk zijn van de prevalentie van een ziekte in de populatie, dat niet correct is. Verschillen in prevalentie van ziektes en verschillen in distributie van ziektes in verschilllende stadia kunnen daarom indicaties zijn voor verschillen van het ontwerp van de studie en studie populatie.

Het hebben van meerdere positieve dieren na de SICCT test was geopenbaard als een risico voor het achterlaten van geinfecteerde dieren. Kuddes met meer dan 1 test positief dier hadden een groter risico om meerdere test positieve dieren te hebben in de toekomst ongeacht of de infectie bevestigd werd of niet met bijvoorbeeld laboratorium testen. Deze resultaten vormden de wetenschappelijke basis voor een verandering in beleid sinds Maart 2018 in Noord Ierland. Kuddes met meer dan 1 test positief dier volgen nu een stricter test beleid dan voorheen.

Hoofdstuk 2 van dit proefschrift richt zich met name op de inspectie voor runder tuberculose in het slachthuis door het richten op risico factoren voor de ontwikkeling van laesies in SICCT test positieve dieren. De risico factoren zijn leeftijd, ras, geslacht, jaar dat het dier werd geslacht, grote van de zwelling van de tuberculine injectie plaats, kudde van herkomst, aantal SICCT test positieve dieren, aantal dieren in de kudde en eerdere test resultaten. Het feit dat deze risico factoren nu bekend zijn assisteren ons om de inspectie in het slachthuis nu beter aan te passen voor dieren in de specifieke categorieën.

Er zijn ook social aspecten die belangerijk zijn voor de eradicatie van runder tuberculose. Op het moment zijn alle aspecten van het runder tuberculose eradicatie programma in Noord Ierland onder de controle van de overheid. Dit gebrek aan directe betrokkenheid van de rundvee sector was de aanleiding voor de studie naar de houding en het gedrag van rundveehouders zoals beschreven in hoofdstuk 3 en 4 in dit proefschrift. De resultaten van deze studie zetten een basis niveau ten opzichte van de huidige houding van veehouders ten aanzichte van de controle van runder tuberculose en biosecurity in Noord-Ierland. De studie concludeert dat er een gebrek is aan de adoptie van runder tuberculose gerelateerde biosecurity door veehouders met name ten opzichte van dassen. Dit resultaat wordt niet alleen gezien op boerderijen die recente uitbraak van runder tuberculose hebben gehad maar ook op boerderij die niet in deze catogorie vallen. Het is ook duidelijk geworden dat veehouders momenteel weining doen om de introductie van runder tuberculose door het kopen van dieren te vermijden. Er is veel scope om dit te verbeteren. Sinds deze studie is uitgevoerd de overheid heeft actief geprobeerd om veehouders aan te moedigen om biosecurity maatregelen toe te passen op hun boerderij. Een ander belangerijk resultaat was dat in het algemeen veehouders voorstanders bleken te zijn van het vaccineren en ruimen van dassen. Vaccinatie kreeg de meeste ondersteuning. Dit is belangerijk omdat sinds de studie was uitgevoerd verschillende das interventie programmas zijn ontworpen en uitgevoerd in Noord Ierland. Veehouders gaven aan dat de financiele implicaties voor alle aspecten van de controle van runder tuberculose een struikelblok is voor hen.

Het is in het algemeen geaccepteerd dat dassen een reservoir kunnen zijn voor runder tuberculose in het Verenigd Koninkrijk en Ierland. De studie beschreven in hoofdstuk 4 van dit proefschrift onderzoekt de associatie tussen dassen en management op rundvee bedrijven met een tuberculose uitbraak. De risico factoren voor een runder tuberculose uitbraak waren onder andere de aanwezigheid van dassen hollen, de observatie van levende dassen op de boerderij en het voeren van magnesium en brokken door het kuilvoer. Dassenhollen die bereikbaar waren voor rundvee waren aanwezig op een groot gedeelte (~83%) van de boerderijen en het merendeel van de veehouders waren goed in het correct identificeren van dassen hollen. De mogelijkheid voor direct contact tussen dieren van buurt boerderijen was reëel en baart zorgen omdat de infectie zich zo kan verspreiden.

Runder tuberculose is een complexe ziekte en er zijn nog veel problemen ten opzichte van de structuur en cultuur van de rundveehouderij in Noord Ierland en de diagnostische testen die worden toegepast. Dit proefschrift heeft enkele van deze problemen geaddresseerd en deze zullen hopelijk, samen met verdere studies, bijdragen aan de controle en eliminatie van runder tuberculose in Noord-Ierland.

Chapter 10

Acknowledgements

Curriculum Vitae

Publications

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Curriculum Vitae

Maria O’Hagan was born Maria Johanna Henrica Vullings on 23th July 1972 in Horst, Limburg, The Netherlands. She completed VWO-B at Boschveld College in Venray and thereafter attended the Faculty of Veterinary Medicine of Utrecht University. After graduation she worked as a veterinary practitioner in mixed veterinary practices in county Donegal, Republic of Ireland and county Tyrone, Northern Ireland up until 2012. In 2012 she joined the Agri-Food and Biosciences Institute in Belfast as a veterinary research officer mainly focussing on bovine tuberculosis research. In 2013 she took up a post as a veterinary epidemiologist in the Veterinary Epidemiology Unit for the Department of Agriculture, Environment and Rural Affairs in Belfast. In this current role her main focus is bovine tuberculosis research alongside other work priorities such as epizootic diseases and disease surveillance.

Alongside a degree in veterinary medicine from the University of Utrecht, Maria holds an MSc in Veterinary Epidemiology and Public Health from the University of London, an MSc in Communication from Queen’s University, Belfast and a Post Graduate Diploma in Food Regulatory Affairs (Veterinary Public Health) from the University of Ulster/University College Dublin.

Publications

O'Hagan, M.J.H., Courcier, E.A., Drew, J. A., Gordon, A.W., McNair, J., Abernethy, D.A., 2015. Risk factors for visible lesions or positive laboratory tests in bovine tuberculosis reactor cattle in Northern Ireland. Preventive Veterinary Medicine Jul 1;120(3-4):283-90

O'Hagan, M.J.H., Matthews, D.I., Laird, C., McDowell, S.W., 2016. Farmer beliefs about bovine tuberculosis control in Northern Ireland. Veterinary Journal 212, 22-26

O'Hagan, M.J.H., Matthews, D.I., Laird, C., McDowell, S.W., 2016. Herd-level risk factors for bovine tuberculosis and adoption of related biosecurity measures in Northern Ireland: A case-control study. Veterinary Journal 213, 26-32

Doyle, L.P., Courcier, E.A., Gordon, A.W., O'Hagan, M.J.H., Menzies, F.D., 2016. Bovine tuberculosis in Northern Ireland: Risk factors associated with duration and recurrence of chronic herd breakdowns. Preventive Veterinary Medicine 131, 1-7.

Walker, D., Fee, S.A., Hartley, G., Learmount, J., O'Hagan, M.J.H., Meredith, A.L., Bronsvoort, B.M.C., Porphyre, T., Sharp, C.P., Philbey, A.W., 2016. Serological and molecular epidemiology of canine adenovirus type 1 in red foxes (*Vulpes vulpes*) in the United Kingdom. Nature 6:36051 | DOI: 10.1038/srep36051

O'Hagan, M.J.H., Menzies, F.D., 2017. Farmer's perception on badger activity. Veterinary Record, doi:10.136/vr.j691

Doyle, L.P., Courcier, E.A., Gordon, A.W., O'Hagan, M.J.H., Stegeman, J.A., Menzies, F.D., 2017. Bovine tuberculosis in Northern Ireland: Quantification of the population disease level effect from cattle leaving herds detected as a source of infection. Epidemiology and Infection 145, 1-11

Lahuerta-Marin, A., Brennan, M.L., Finney, G., O'Hagan, M.J.H., Jack, C., 2018. Key actors in driving behavioural change in relation to on-farm biosecurity; a Northern Ireland perspective. Irish Veterinary Journal 71:14

O'Hagan, M.J.H., Stegeman, J.A., Doyle, L.P., Stringer, L.A., Courcier, E.A., Menzies, F.D., 2018. The impact of the number of tuberculin skin test reactors and infection confirmation on the risk of future bovine tuberculosis incidents; a Northern Ireland perspective. Epidemiology and Infection 146(12), 1495-1502