Epidemiological Investigation of Chronic Bovine Tuberculosis Herd Breakdowns in Northern Ireland

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EPIDEMIOLOGICAL INVESTIGATION OF CHRONIC BOVINE TUBERCULOSIS HERD BREAKDOWNS IN NORTHERN IRELAND

Epidemiologisch Onderzoek naar Chronische Runder Tuberculose in Noord Ierland

(met een samenvatting in het Nederlands)

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Chapter 1

Introduction

Tuberculosis is an infectious disease that can affect practically all vertebrates and is caused by acid-fast bacilli of the genus *Mycobacterium.* Classically it is defined as a chronic debilitating disease; however, it occasionally assumes an acute and rapidly progressive course. Lesions and clinical signs are generally similar across different species. Three main types of tuberculosis are recognised: human, bovine and avian with *Mycobacterium bovis* being the main cause of bovine tuberculosis (bTB) in cattle [1]. It is a highly adapted pathogen with a world-wide distribution and, in several countries, it remains an economically important infectious disease of cattle and other domesticated, feral and wild animal populations, including badgers (*Meles meles*), possums (*Trichosurus vulpecula)*, deer (family *Cervidae*), goats (*Capra hircus*), sheep (*Ovis aires*) and camelids (family *Camelidae*) [2].

There are several ways in which bTB spreads between cattle including horizontal spread by inhalation or ingestion and vertical transmission. It is however widely accepted that aerosol is the most important infection route with very low numbers of bacteria required for establishing infection [3, 4]. In Northern Ireland (NI), first statutory attempts at control of bTB began in 1949 with a compulsory programme introduced in 1959. However, despite the current national bTB eradication programme of annual test surveillance along with enhanced risk testing (with removal of test positive animals from the population, curtailing infection transmission) and abattoir surveillance, bTB has remained endemic in the NI cattle population [5, 6].

The mainstay surveillance test used in NI (in compliance with European Union (EU) law - 64/432/EEC) to determine presence of *M. bovis* infection is the bTB skin test, which in the British Isles is the common name for the Single Intradermal Comparative Cervical Tuberculin (SICCT) test. Sensitivity of the SICCT test has been reported in many publications, both as meta-analyses and individual studies. In a review based on work from a number of different countries, sensitivity of the SICCT test was estimated to be 50% with wide credible intervals (95% CrI: 26-78) [7]. Individual studies from the United Kingdom (UK) and Republic of Ireland (ROI) have in general reported higher sensitivity values for the SICCT test at 88.6% (95% CrI: 85.4-92.2) in Northern Ireland [8], 66% (95% CI: 52-80) in Great Britain (GB) [9] and ROI 56.8% (95% CrI: 52.9-60.8) [10]. Based on studies carried out in populations of bTB free cattle, specificity of the SICCT test has been estimated to be very high with a median value of 99.5% (Range: 78.8-100%) [11] which concurs with other studies [7, 8, 12].

Mycobacterium bovis as a zoonosis

Mycobacterium bovis can enter human hosts by ingestion, aerosol inhalation or direct contact with mucous membranes and skin abrasions. Tuberculosis due to *M. bovis* is a rare infection in humans within the EU (European Union), with 170 confirmed cases in humans reported in 2018 (93 of these cases were in individuals of EU origin) [13]. In industrialised countries such as UK and ROI, where milk pasteurization is widespread or compulsory, bTB no longer poses a risk to the urban population despite a relatively high bTB incidence in the national cattle herds [14]. By contrast, in developing countries *M. bovis* infection may still constitute a major threat to public health, with the fraction attributable to human TB cases largely unknown, but likely to be much higher than in industrialised countries and speculated to account for as many as 10-15% of new human cases [14].

The Northern Ireland Cattle industry

In June 2018, the NI cattle population was approximately 1.6 million (including 247,000 beef cows and 314,000 dairy cows) in approximately 24,000 active herds [15]. The livestock and livestock products industry in NI is export dependent with 2018 external sales worth in excess of £1.79 billion to the local economy [16].

The structure of the NI cattle industry is unique in many ways. One important feature about NI livestock farming is the fact that there is very high cattle density (112 cattle per km^2), one of the highest in Europe [17]. Another important feature is the degree of fragmentation of farms with 60% of the herds having multiple premises [5]. These farms, even though fragmented, are treated as a single epidemiological unit and farmers can move animals at will within the unit. Also the majority of NI cattle herds do not have adequate facilities to finish fatstock (keep animals on their own premises for the full beef production cycle), instead making use of livestock markets [5].

Bovine Tuberculosis in Northern Ireland

DAERA (Department of Agriculture, Environment and Rural Affairs) has an EU Commission approved bTB eradication programme which ensures compliance with the EU Trade Directive 64/432/EEC. The 2019 NI bTB eradication programme cost on an annual basis £35 million. However it is subject to yearly fluctuations determined mainly by the amount of compensation payment required for the purchase of bTB reactor cattle, which is dependent on disease incidence [16].

In 1949, NI began a voluntary statutory bTB eradication programme. The pillars of this programme were based on tuberculin testing of cattle, removal of reactor animals, financial compensation, movement controls and cleansing and disinfection of the infected premises. Herds in which cattle tested negative were given an attested status. These essential components, instigated in 1949, remain essentially the same to this day. At the start of the programme, it was estimated that animal bTB incidence was about 25% [6]. By May 1959, 55% of the NI cattle population were included in the voluntary scheme, with this moving to a compulsory scheme in September 1959. By May 1960, 898,946 cattle (93% of cattle in the country) were either part of fully bTB attested herds or part of herds on their way to achieving attested status [6]. Rapid progress in the eradication of bTB was made reducing the disease to very low levels, which lead to the introduction of biannual bTB testing in 1965 and then triannual bTB testing in 1971. Indeed, it appears that the early 1970s were a pivotal moment in the NI bTB story, where a possible window of opportunity to eradicate bTB was lost both in terms of the bTB programme and more fundamentally; a world of change to the prevailing agricultural systems by accelerated intensification of systems along with UK and ROI joining the European Communities on the 1st January 1973.

Up to 1971, bTB eradication had been reported as a rapid, generally smooth and straightforward exercise, with bTB being thought of as virtually a disease of the past [6, 18]. However, at that point, worrying evidence began to emerge of a bTB resurgence, which threatened further progress and indeed set the scene for the following 50 years [6]. Incidence levels of bTB increased during the early 1970's rising from 0.54% in 1971 to 2.54% in 1976 (DAERA, unpublished data), leading to the emergence of a state of attrition between the disease, stakeholders and the eradication programme. Reasons given at the time for this inflexion point in the bTB eradication programme were:

1. The introduction of biannual testing in 1965 and its replacement with triannual testing in 1971 led to a surveillance testing programme that was too infrequent to prevent increased transmission rates.
2. A shortage of veterinary surgeons in the early 1970s meant that even the triannual testing was not being implemented as required by the programme [6], further increasing the test interval and allowing more opportunity for inter-herd spread and also leading to inadequate investigation of bTB outbreaks.
3. Changes in cattle demographics where there was a 25% increase in cattle numbers between 1972 and 1975 [6] combined with increasing herd sizes (average dairy herd size increased from 19 to 34 cows between 1974 and 1986) [6]. Increasing herd sizes meant that removal of all infected cattle from herds using the SICCT test became increasingly difficult due to imperfect test sensitivity.
4. The quality of application of the SICCT test was also starting to be questioned during the 1970's with commentary at the time from the CVO (Chief Veterinary Officer) noting increased reports of testing "not being carried out properly" [6]. Criticism was also levelled at the Government Veterinary Division due to its opposition to close supervision of Private Veterinary Practitioners (PVP) and its implementation of a decentralised bTB control strategy which led to varied standards of programme implementation across the divisional veterinary offices [18].
5. The renting of ground between April and November, a traditional practice known as "conacre" became much more accessible to farmers. Furthermore, improvements in cattle transportation facilitated more dispersal of cattle to a greater variety of rented farms, which could be a significant distance away from the main farm. This change also allowed a greater contact between cattle from a variety of herds of different disease risk levels.
6. An issue pertinent to the time was importation of cattle (both legal and illegal movements) from ROI. Between 1971 and 1976, large numbers of cattle imports were blamed for increased bTB incidence [19].

In 1982, annual bTB testing was reintroduced and this appeared to have an effect on bTB levels with herd incidence levels reducing to 1.25% in 1987, the lowest level since the early 1970s. However, the bTB incidence rose steadily from 1987 and a 1990 Departmental review introduced an "enhanced eradication programme" but bTB levels continued to rise throughout the 1990s. BSE control measures may have diverted attention/resources from bTB controls during this period. This bTB trend continued until 2002 when, after the 2001 Foot and Mouth disease incursion into NI (accompanied by a suspension in TB testing for several months in 2001), bTB reached a peak herd incidence of 9.93%. [6, 20]. The NI incidence of bTB (and other countries of the British Isles) during 1995 to 2015 are detailed in published papers [21, 22] but infection levels were higher at the end of the first study period (2010) than in 1995 (unadjusted herd incidence was 4.4% in 1995 and 6.0% in 2010, respectively) [21]. A fluctuating pattern was observed over the last decade, which was underpinned by an insidious upward trend in bTB herd incidence culminating in an annual bTB herd incidence in 2017 of 9.61%.

Since this peak, bTB herd incidence has fallen slightly but any trend has been confounded due the cessation of some surveillance measures through the impact of the COVID-19 pandemic. The 2020 NI annual bTB herd incidence was 8.44%.

From 2000 onwards, changes to the bTB eradication programme were implemented to try and ensure more effective control of the infection in the cattle population. These measures included an automatic ban on cattle movements out of herds with overdue bTB tests in 2004, introduction of interferon gamma (IFNG) testing in 2007 as a parallel test to enhance SICCT test sensitivity in some bTB herd breakdowns [23, 24], removal of animals with two consecutive inconclusive bTB SICCT test results in 2010 [22, 25, 26], use of severe interpretation of the SICCT test in all restricted herd tests and the requirement for two consecutively negative SICCT test herd tests to derestrict bTB herd breakdowns in herds with multiple SICCT reactors. Also introduced were broader initiatives such as introduction in 2016 of a PVP (Private Veterinary Practitioner) contract, which aimed to improve overall quality of bTB testing [23] by providing feedback to individual vets and establishing formal methods to assess performance.

Furthermore in 2013, the then Agriculture and Rural Development Minister announced her intention to establish a Government / industry partnership to develop a long-term strategy to eradicate bovine bTB from the NI cattle population. An independent strategic partnership was set up and their comprehensive report was published in 2016, which proposed a bTB strategy and an implementation plan targeted at a progressive and sustained reduction in both the levels of disease and the cost of the bTB eradication programme [27].

Bovine Tuberculosis surveillance in Northern Ireland

Bovine TB is the most complex and difficult multispecies endemic animal disease currently facing government, the veterinary profession and the farming industry in the UK and ROI. Despite sustained and costly implementation of eradication programmes, it has not been eradicated from either country [2].

Current bTB eradication measures in NI require all cattle over 6 weeks-old to be tested annually for the infection using the SICCT test. This test relies on a delayed-type hypersensitivity response of the animal to the injection of tuberculin [28]. With this test, 0.1ml of avian and bovine tuberculin are injected intradermally in the neck region, at sites clip-marked and approximately 12.5cm apart. The test involves an *in vivo* cell mediated response to the tuberculin and is read by comparing increase in skin-fold thickness at the avian and bovine sites 72 hours post injection. The test can be interpreted in different ways based on the cut off values applied. Standard interpretation positive refers to the situation where the bovine reaction is ≥4mm and exceeds the avian reaction by >4mm. Severe interpretation positive refers to the situation where the bovine reaction is > 2mm and exceeds the avian reaction [10, 28]. Herd level testing is undertaken by private veterinary practitioners or government veterinary officers. The bTB programme, including all test results, herd and animal details and cattle movements are all recorded on a national database, APHIS (Animal and Public Health Information System) [29]. In terms of compliance to bTB herd testing of the 22,295 herds tested in 2020 less than 1.5% had a herd test which was not completed within three months of the due date (DAERA, unpublished data).

Cattle that are SICCT test positive are compulsorily removed and slaughtered. At a herd level, confirmation of bTB infection occurs when a SICCT test positive animal is disclosed with visible bTB like lesions at slaughter or there is disclosure of greater than one SICCT test positive animal independent of lesion status. Confirmation can also occur when lymph nodes from an animal are confirmed as histological and/or bacteriological positive for bTB on laboratory examination.

Officially tuberculosis free (OTF) is a statutory declaration of a herd's freedom from bTB infection and allows it to trade with other herds. If a herd has bTB confirmed, its OTF status is changed to Officially Tuberculosis Withdrawn (OTW), with this only reverting back to OTF if its statutory testing obligations have been fulfilled. The period of time for which the herd has the OTW status applied is known as the breakdown period and restricts the herd from trading cattle with other herds. If the positive test is a disclosing test (test at which an OTF herd discloses SICCT test positive animals) and infection has not been confirmed by the presence of visible bTB-like lesions in reactors at slaughter or multiple SICCT test positive animals, then sampling of lymph nodes and other appropriate tissues is carried out from these animals. These samples undergo histological and bacteriological laboratory testing in order to provide confirmation of infection. It has been reported that the sensitivity of standard post mortem examination is low (47%) for detection of tuberculous-like lesions [30]. This means that a negative post-mortem in reactor cattle is not accepted as evidence that the animals do not have infection. Cattle can also disclose tuberculous lesions at routine slaughter meat inspection (LRS). These samples also undergo histological and bacteriological laboratory testing in order to provide confirmation of disease. On disclosure, herds are restricted from moving cattle (except to direct slaughter) and undergo a series of short interval herd tests (usually 60 days apart). Two negative consecutive herd tests must be achieved before removal of these cattle movement restrictions allowing the herd to resume inter-herd trading of cattle.

All herds contiguous to the disclosing herd and those traced as having moved animal to or received animals from the disclosing herd are identified and risk herd tests are set, subject to risk assessment by local veterinary staff. This means that the shortest possible breakdown period of bTB confirmed herds is 120 days. If bTB is not confirmed, then the shortest possible breakdown period is 60 days. In NI, the median length of a bTB breakdown period (for restrictions ending in 2015) was 192 days (IQR: 144-268), which is the longest median duration of any jurisdiction in the British Isles. In terms of prolonged restrictions (>550 days) ending in 2015 the percentage of prolonged restrictions was highest in the High Risk Area (HRA) [22] of England (6.4 per cent), Wales (5.1 per cent) and Northern Ireland (4.3 per cent) and consistently less over the study period in Edge Area and Low Risk Area (LRA) of England (2.3 and 0.93 per cent, respectively) and in ROI (1.4 percent) [22].

The other important elements of NI bTB surveillance are the IFNG (interferon gamma) test and post mortem inspection of all cattle routinely passing through abattoirs. In 2002, the European Commission approved the IFNG test (EC/1226/2002 amending Annex B of directive 64/432/EEC) to be used as a complementary test to the SICCT test. Since 2004, DAERA have used the IFNG test alongside the SICCT test (as parallel tests) in bTB infected herds. This provides a higher overall test sensitivity, thus removing more infected animals, hence aiming to reduce the rate of bTB breakdown recurrence in the herd. The IFNG test has superior sensitivity (88-94%), but inferior specificity (85-98%) making it more prone to disclosing false positives [11].

All cattle slaughtered through NI abattoirs are inspected for gross tuberculous-like lesions as required under EC Directive 854/2004. This acts as an additional surveillance system where any disclosed tuberculous-like lesion is sampled and sent for laboratory examination. Herds with a suspected tuberculous-like lesion are immediately restricted from moving cattle off their premises to markets or other herds and will not regain their OTF without full compliance to the DAERA bTB disease programme. In NI during 2018, 2.49 animals per thousand undergoing post-mortem examination and disclosing a tuberculous-like lesion were confirmed; a confirmation rate of 60% [16].

Bovine Tuberculosis in Europe

Within the EU, member states can be OTF, non-OTF or have regions within their jurisdictions OTF/non-OTF [13]. In 2018, OTF regions of 21 MS (member states) recorded 172 (0.015%) bTB infected herds, which was consistent with previous years. In contrast non-OTF regions in 11 MS reported 18801 (1.93%) bTB positive herds. Of these 18801 bTB herd breakdowns, 10359 (55%) were in the UK (United Kingdom), 5573 (30%) in ROI, 2384 (13%) in Spain, with the remainder found across Italy, Portugal, Bulgaria and Greece. Croatia and Romania reported *Mycobacterium* complex (MTC) and *M. caprae* infected herds at a low level. In 2018, five countries had EU co-financed eradication programmes, ROI, Italy, Portugal, Spain and the UK [13].

In general, continental European countries do not have the same problem as Britain (except Scotland which is regionally OTF) and ROI but practices in relation to bTB control are changing. The use of SICCT testing to screen for disease at herd level, followed by depopulation of the herd post disease detection was previously a common policy in France. However this practice, both in France and on a wider continental context has more recently been seen as a less attractive option due to economic and welfare concerns [17, 31]. Another difference between continental Europe and the countries of the British Isles is the testing method. The single intradermal test (SIT) / cervical intradermal test (CIT) used in continental Europe involves inoculation of *M. bovis* derived purified protein derivative (PPD) as a single injection to determine increases in skin thickness indicative of infection [11, 17, 31]. In UK and ROI due to environmental *M. avium* complex reducing specificity, the comparative SICCT test is used. Reasons as to why the British Isles has such a significant bTB problem relative to continental Europe could be related to several factors such as high cattle densities in comparison to other European countries [17], trade as a significant characteristic of cattle farming [17, 32] and highest average recorded density of badgers compared to any other country in Europe [33].

Objectives and outline of the dissertation

The current NI bTB eradication programme involves annual SICCT testing, extensive computerized tracing, short interval testing of bTB breakdowns and herds contiguous to them, and compulsory slaughter of SICCT test reactors. However, failure to eradicate bTB through this compulsory programme, which has been in place for nearly sixty years, has been attributed to a wide range of factors [5]. There are undoubtedly significant socioeconomic and political factors which impact upon disease eradication [6] coupled with ecological and epidemiological constraints present in these islands relative to other parts of Europe, which may interact to diminish the potential of the programmes to achieve eradication [17]. Some of the current issues identified as having a major impact on eradication include heterogeneity of diagnostic testing approaches and the presence of an abundant wildlife reservoir of infection. The nature, size, density and network structure of cattle farming are also factors which impact eradication efforts, as are the potential effects of *M. bovis* strain heterogeneity. Other factors identified are the possible impacts of concurrent endemic infections on the disclosure of truly infected animals; climatological differences and change coupled with environmental contamination [17].

One area of importance to NI in relation to bTB and a subject of focus in this dissertation is how certain herds seem to have a much greater propensity to develop prolonged and recurrent bTB breakdowns; with the term "chronic bTB herd" being applied to them. The problem with these herds is that they generate large numbers of infected animals, thus are a significant financial burden to the government in compensation payments, and present enhanced risk to any other herds epidemiologically linked to them. This subject area has been debated for many years with an initial internal DAERA report (Abernethy to Animal Health and Welfare Group, dated 01/04/2005) defining such herds as those which disclosed SICCT test reactors in at least three tests within two calendar years. Using this definition, it was shown that the number of chronic bTB herds was increasing (1996-2000 – 18% vs 2001-2003 – 27%) and that in the period 2001 to 2003, these 27% of herds contributed 56% of the total number of SICCT test reactors. Chronic bTB herds were also highlighted in the TB Strategic Partnership report as an area requiring focused research, to provide possible solutions to this long term problem [27]. Analysis of the risk factors associated with chronic bTB breakdowns have been researched in both GB and the ROI. These studies investigated how risk factors such as number of SICCT test reactors [34, 35], herd size [34, 36, 37], herd bTB history [34, 35], type of herd [38], purchase patterns [38, 39], husbandry practices [39] and local farm environment [38, 39] impacted upon chronic bTB herd breakdowns.

Chronic bTB herds – What are they?

The NI bTB programme deals with approximately 2000 bTB herd breakdowns per year and natural variation will mean that there is a range of responses; from short duration incidents which will contain only one SICCT test reactor animal to those which carry on for years generating many reactors. Does this mean that the idea of chronic bTB herds could simply be a phenomenon of natural variation or is there something different about these herds which means that they require special attention within the bTB eradication programme? Reductionism applied to an area such as bTB is always attractive as it provides a methodology for investigation of complex entities; that is, simplify to quantitative units so that statistical methods can be applied [40]. However reductionism risks oversimplification of a process, decreasing it down to a point where it can be potentially disassociated from the phenomenon it is trying to explain [40]. This is a risk with the idea of investigating something defined as chronic bTB herds, as we could be placing definitions on something which exists naturally as part of simple statistical variation linked to any complex biological system. Indeed, with a concept such as chronic bTB herds, a certain vision is required to ascertain something which seems obvious to many in hindsight; the fact that certain herds seem to constantly disclose disease while others do not. This vision was articulated succinctly in Griffin et al. (1993) [38] when the author stated that a feature of the disease is its chronic nature in certain herds. This scientific investigative process into chronic bTB herds across the British Isles is still ongoing.

This dissertation is based on six separately conducted studies; as outlined below.

Chapter 2 focuses on the risk factors which determine how long a herd will remain OTF after completion of all official testing related to that bTB herd breakdown. The study implements a survival analysis design using Kaplan-Meier survival estimates and a Cox proportional hazards model to demonstrate the significant associations. Practically it is useful to gain an understanding of which risk factors are likely to contribute towards a bTB herd breaking down earlier after the previous disease incident as this type of information is useful to policy makers and individuals involved directly in implementation of disease control measures.

Chapter 3 is a study focused directly on chronic bTB herds and provided definitions within the NI context of prolonged and recurrent breakdowns. The purpose of this paper was to use data within the national database, APHIS, to look at risk factors associated with chronic bTB herds and also to provide published definitions for prolonged and recurrent bTB breakdowns. The providing of these definitions allowed further work to be carried out on the subject area, specifically Chapters 6 and 7.

Chapter 4 takes a risk factor which discloses in many bTB studies, namely cattle movement, and looked at how it impacted on disease levels from a NI aspect. Determination of the proportion of bTB herd breakdowns attributed to a herd purchasing infected animals had not been previously quantified using NI data. We used a case–control study design to account for the infection process occurring in the disclosing bTB herd breakdowns to which animals were moved. This allowed for the calculation of the Population Attributable Fraction (PAF) of disease in these herds attributable to cattle received, which in turn meant it was possible to quantify the overall effect of this risk factor in a NI context.

Chapter 5 was a collaborative project [22] between GB, NI and the ROI which aimed to standardise statistical measurements of disease between the different jurisdictions and thus allow comparison of disease trends in the period from 2003 to 2015. It was very important that NI was able to contribute to this project as it provided a benchmark for comparison of its national programme with other countries who are aiming towards the same goal of disease eradication. The author of this thesis represented NI in this project and collaborated with the country representatives under direction from CVERA (Centre for Veterinary Epidemiology and Risk Analysis) at UCD (University College Dublin), ROI.

Chapter 6 was a paper developed using Chapter 3 as a basis for its definitions. This study used an epidemiological questionnaire which gathered information from NI bTB herd breakdowns to determine which farm management factors were associated with long duration bTB herd breakdowns. This work was a natural progression from Chapter 3 where it looked at the actual farm level factors associated to prolonged bTB herd breakdowns rather than the data stored in the APHIS database and aimed to provide more practical associations between chronic disease and farm management practices.

Chapter 7 was a paper developed using Chapter 3 as a basis for its definitions. This study used an epidemiological questionnaire which gathered information from NI bTB herd breakdowns to determine which farm management factors were associated with recurrent bTB herd breakdowns. Unlike the study presented in Chapter 3, this work looked at the farm management level factors associated with chronic recurrent bTB herd breakdowns using the epidemiological investigations carried out on farm post disease confirmation.

Chapter 8 is a general discussion in relation to bTB in NI, which focuses on the impact of research conducted in this thesis.

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Chapter 2

Bovine tuberculosis in Northern Ireland: Risk factors associated with time from post-outbreak test to subsequent herd breakdown

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Bovine tuberculosis in Northern Ireland: Risk factors associated with time from post-outbreak test to subsequent herd breakdown

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Abstract

Compulsory bovine Tuberculosis testing has been implemented since 1959 in Northern Ireland. Initial rapid progress in the eradication of the disease was followed by a situation where disease levels tended to fluctuate around a low level. This study explores recrudescence of bovine tuberculosis (bTB) in Northern Ireland herds by assessing risk factors associated with time from the six-month post-outbreak skin test until a further herd breakdown. Bovine herds (n = 3377) were recruited in 2002 and 2003 and their survival analysed using Kaplan-Meier survival estimates and a Cox proportional hazards model, with follow-up extending to August 2008. Exclusion criteria applied for study entry were bTB infection in a contiguous herd, changing of post restriction test to one of a higher risk status or chronic infection. Chronic infection was defined as any situation where disclosure preceded the post-outbreak test by two years or more. The application of these exclusion criteria meant that herds recruited to the study were largely cleared of infection and not directly contiguous to other infected herds. Of the 3377 herds, 1402 (41.5%) suffered a further herd breakdown before the end of follow-up. Median survival time was 582 days (interquartile range = 336 – 1002 days). Breakdown severity (defined as the number of Single Intradermal Comparative Tuberculin Test (SICTT) reactors at disclosure test), local bTB prevalence, herd size and type were identified as significant risk factors ($p<0.05$), as was the purchase of higher numbers ($n>27.38$ per year) of cattle. Consistent with other studies this work shows bTB confirmation to not be predictive of a future herd breakdown. This work shows bTB history as not being a risk factor for a future breakdown. This result could be reflective of the exclusion criteria used in the study, which may have selected for incidents where historical status was of less importance.

Key words: Epidemiology, *Mycobacterium bovis,* breakdown severity, Kaplan-Meier, Cox proportional hazards, cattle.

Introduction

Tuberculosis in cattle caused by *Mycobacterium bovis* has a worldwide distribution. Eradication has been largely successful in developed countries, particularly those with no significant wildlife reservoirs (Abernethy et al., 2006). An eradication scheme was introduced in Northern Ireland in 1959 but bovine tuberculosis remains endemic. Peak incidence (new cases) occurred during the spring of 2003 with a herd incidence of 10.20% and animal incidence of 0.99%. From 2003 to 2007 Northern Ireland bTB levels dropped yearly to reach a consistent level of herd and animal incidence below 6% and 0.60% respectively. This steady state situation remained until autumn 2011 when an increase in both herd and animal incidence reached levels of 7.32% (herd incidence) and 0.66% (animal incidence) in 2012. Post the peak levels a consistent reduction in bTB levels have been documented during 2013 (www.dardni.gov.uk; accessed 16/12/2013). These figures are placed in context in Abernethy et al. (2012) where bTB trends are described for the UK and Republic of Ireland in the period 1995 to 2010. It describes a stable situation of very low bTB prevalence in Scotland and over most of the period a rising prevalence in England and Wales. The prevalence in the Republic of Ireland declined while Northern Ireland experienced both a rise and fall.

All cattle in Northern Ireland are individually identified and have both lifetime movement and tuberculosis test histories recorded. BTB surveillance of all herds is conducted annually using the Single Intradermal Comparative Tuberculin Test (SICTT) with test frequency increasing when bTB is suspected or proven, or where contiguous herds suffer a breakdown. Upon confirmation of bTB, a herd must enter a cycle of short-interval ('restricted') tests at sixty day intervals and movement restrictions are only lifted after the herd achieves two consecutive clear SICTT tests. When this herd restriction is removed, freedom to trade is restored but a post-outbreak test after six months is required. If the six month post-outbreak test is clear and subject to no other risk factors such as traced or contiguous infection it is placed back onto a yearly test cycle. Details of the Northern Ireland bTB control and eradication programme are given in Abernethy et al. (2006). The aim of this study was to investigate selected factors associated with time from the post-outbreak test to subsequent herd breakdown.

Risk factors associated with bTB herd breakdowns have been extensively researched. These factors include presence of a wildlife source (Biek et al., 2012; Griffin et al., 1996; Ó Máirtin et al., 1998; Eves, 1999; Griffin et al., 2005; Reilly and Courtenay, 2007), presence of bTB in contiguous herds (Griffin et al., 1996; Denny and Wilesmith., 1999; White et al., 2013), environmental survival of *M. bovis* (Scanlon and Quinn., 2000; Ramírez-Villaescusa et al., 2010), previous bTB history (Olea-Popelka et al., 2004; Carrique-Mas et al., 2008; White et al., 2013), movement of animals (Carrique-Mas et al., 2008; Johnston et al., 2005; Ramírez-Villaescusa et al., 2010), severity of bTB breakdown (Olea-Popelka et al., 2004; Wolfe et al., 2010; Karolemeas et al., 2011 Gallagher et al., 2013), herd type (Griffin et al. 1993; Ramírez-Villaescusa et al., 2010; Alvarez et al., 2012), herd size (Griffin et al., 1996; Green and Cornell, 2005; Brooks-Pollock et al., 2009, Mill et al., 2011; Gallagher et al., 2013) and bTB confirmation ((Olea-Popelka et al., 2004; Abernethy et al., 2010; Wolfe et al., 2010; Karolemeas et al., 2011). Confirmation plays an important role in the Northern Ireland bTB testing programme. If bTB is confirmed in a herd the shortest possible restricted period is 120 days while with non-confirmation the shortest possible restricted period is 60 days.

Materials and methods

Study design and study population

A retrospective cohort study was undertaken. The study population comprised all herds with a negative post-outbreak test between January 1st 2002 and January 31st 2004. This period was chosen so as to avoid the disruption to normal bTB testing patterns associated with the 2001 foot and mouth epidemic. Herds were recruited to the study on the date they completed the clear post-outbreak test. On completion of a clear post-outbreak test these herds had carried out at least two negative herd tests and disclosed no bTB for a period of at least six months. Thus herds recruited to the study should fall into a subset where bTB internal to the herd is minimised. Exit from the study occurred when the herd suffered a subsequent bTB outbreak event, defined as the first positive SICTT following a negative post-outbreak test or the discovery of a confirmed TB lesion at routine slaughter (LRS). The disclosure date of the positive SICTT or LRS was considered to be the date of the bTB outbreak event, and all variables were related to this time-point. End of follow-up was 15th August 2008 and any herd that had not suffered a herd break down at this point exited the study.

Chronic breakdown herds (herds where bTB disclosure preceded the post-outbreak test by two years or more) or those located contiguous to another bTB infected herd were excluded from the study. Also excluded from the study were those herds with tests initially set as post-outbreak tests and later re-categorised to a higher risk status.

Data collection and variable definitions

The identification, movement and test history of all individual bovine animals was extracted from the Animal and Public Health Information System (APHIS) run by the Department of Agriculture and Rural Development for Northern Ireland. Variables included breakdown severity, local prevalence of bTB, herd size, herd type, purchase intensity, confirmation, and bTB history of the herd. Breakdown severity was based on the number of standard reactors (0, 1, 2 - 3, 4 - 8, > 8) as recommended by O'Keeffe et al. (1998) with 0 reactors representing the situation where there is detection of a suspect lesion at slaughter and the first herd level test in a breakdown returns no skin reactors. Local prevalence for bTB was defined as the percentage herd prevalence in the local district council area at the date of the post-restriction herd test. Herd size was the number of cattle tested at the first herd level test in the breakdown. Herd type was either non-dairy or dairy with the latter herds being in possession of a milk licence. Purchase intensity was calculated as the number of cattle purchased into a herd after derestriction divided by the period the herd remained disease free or reached the end of follow up. BTB confirmation was based on finding visible lesions in SICTT reactor animals or obtaining a positive histological or bacteriological examination from a slaughtered animal. Herds were classified as having a history of TB if a SICTT reactor or confirmed LRS occurred in the two years prior to the breakdown.

Data analysis

Microsoft Access^{TM} was used for all data manipulation and STATA/IC 10.0 (Stata, College Station, TX) for data analysis. All continuous variables were categorised so as to create categories containing approximately equal numbers of observations. Relevant summary statistics were calculated for each variable. Relationships between variables were assessed initially without attaching survival data using Spearman's Rank correlation. Determining relationships at this level helped to give more understanding to the eventual survival analysis results. Kaplan-Meier (KM) plots were produced for each variable and logrank tests used to determine whether time to subsequent herd breakdown differed between the levels of each variable (Clark et al., 2003). Univariable and multivariable Cox proportional hazards regression models were used to identify factors significantly associated with time to subsequent herd breakdown. Univariable Cox proportional hazard models were created for each variable and the validity of the proportional hazard assumption assessed in two ways. The first was to visually determine whether the plots of -log(-log) survival lines were parallel (Bradburn et al., 2003). The second was to incorporate each variable into the model as a time-varying covariate (Dohoo et al., 2003). Following univariable analysis all variables were included in a multivariable Cox regression model using a forward stepwise method. All combinations of two-way interactions were assessed between the significant variables in the final model by forcing these terms into the model. The likelihood ratio test was used to assess whether or not their inclusion improved the overall fit of the model. For all analyses a p-value cut off point of 0.05 was considered significant. The final model fit produced as a result of this process was evaluated graphically using a plot of its cumulative hazard function against the Cox-Snell residuals (Dohoo et al., 2003). The power (and indeed in some cases validity) of survival analysis is related to the number of events rather than the number of participants (Bradburn et al., 2003). Simulation work has suggested that at least ten events need to be observed for each covariate considered, with anything less leading to problems (Bradburn et al., 2003). In the current study the maximum number of covariates included in the model at any point was 19, so that 190 events (bTB breakdowns) would be required for the study to have sufficient power. As 1402 events were included in the study it is assumed that the study had sufficient power.

Results

Time from post-outbreak test to subsequent herd breakdown

Of the 3377 herds included in the study, 1402 (42 %) experienced a breakdown before the end of the follow-up period. Table 1 shows how these 3377 herds and the 1402 related bTB incidents are distributed across each exposure level of the study variables. Median length of time to breakdown (582 days (IQR 336 – 1002)) for herds disclosing a bTB event was significantly less than the median length of time in the study for those herds with no bTB event (1679 days (IQR 1506 – 1884)) (Kruskal-Wallis equality-of-populations rank test $\chi 2 = 2103$; $p < 0.001$).

Kaplan-Meier curves for six of the study variables are presented in Figures 1 to 6. Median time to subsequent herd breakdown decreased significantly with increasing breakdown severity (logrank $p < 0.05$; Figure 1), local bTB prevalence (logrank $p < 0.05$; Figure 2), purchase intensity (logrank p = < 0.05; Figure 3) and herd size (logrank $p < 0.05$; Figure 4). Median time to subsequent herd breakdown was significantly less for dairy than non-dairy herds (logrank $p < 0.05$; Figure 5) and for herds in which bTB was confirmed as opposed to those in which it was not confirmed (logrank $p < 0.05$; Figure 6). There was no significant difference in median time to subsequent breakdown of herds with or without a history of bTB (logrank $p = 0.06$).

An assessment of the variables in which basic correlations were examined without taking any account of survival data was carried out. Correlations shown to be significant were herd size and herd type (Spearman's rank correlation coefficient = 0.4372; $p<0.001$); bTB confirmation and breakdown severity (Spearman's rank correlation coefficient = 0.5916; $p<0.001$) along with herd size and bTB history (Spearman's rank correlation coefficient = 0.1642; $p<0.001$).

Factors associated with time from post-outbreak test to subsequent herd breakdown

All variables except for bTB history were significant in the univariable Cox regression models (Table 2). The final model (Table 3) contained the variables herd size, herd type, local prevalence, breakdown severity and purchase intensity. The p-values for each categorical level of the variables given in the tables are compared to the reference level of that variable. Time to subsequent breakdown was significantly less for dairy herds than non-dairy herds (Hazard Ratio (HR) 1.31, 95 % CI 1.16 - 1.49) while time to subsequent breakdown decreased significantly with increasing herd size, breakdown severity and local bTB prevalence. Rate of subsequent breakdown was 3.17 times higher in herds with more than 180 animals compared to herds of 30 animals. For breakdown severity there was no significant difference (p = 0.582) in herd breakdown rate between zero (i.e. an LRS followed by a clear herd SCITT) or one reactor, but thereafter, rate of herd breakdown increased with increasing number of reactors. Similarly, there was no significant difference in herd breakdown rate between a local bTB prevalence of less than 9 % or 9% to 12 % (p = 0.766), but thereafter breakdown rate increased significantly with increasing local prevalence. Purchase intensities of between 0.73 to 27.38 animals per year had a similar effect on the rate of herd breakdown (HR 0.88 (95 % CI 0.73-1.06) – HR 0.99 (95 % CI 0.83-1.18)), while a purchase intensity relative to this group of > 27.38 animals per year significantly increased rate of herd breakdown (HR 1.23, 95 % CI 1.03 - 1.49). No two-way interactions were significantly associated with time to subsequent herd breakdown.

Discussion

This study and that of Olea-Popelka et al. 2004 are the only studies of which the authors are aware that look at *time* to herd breakdown, rather than simply determining factors associated with *presence* of a breakdown following a post-breakdown herd test. While many studies have investigated factors associated with herd breakdowns only a few have looked at the impact of particular test categories, such as this paper where the post outbreak test is used (Green and Cornell, 2005; Carrique-Mas et al., 2008). Determination of the risk factors associated with time from post-outbreak test to subsequent herd breakdown has potential to assist in formulation of bTB control policies. We found herd size, breakdown severity, herd type, local prevalence and purchase intensity to be significantly associated with time to subsequent herd breakdown, with hazard of breakdown increasing with increasing herd size, breakdown severity, local bTB prevalence and purchase intensity. In addition, dairy herds experienced a subsequent outbreak significantly sooner than non-dairy herds.

Increasing herd size has consistently been shown to be associated with increased risk of herd breakdown (Griffin et al., 1996; Green and Cornell, 2005; Brooks-Pollock et al., 2009, Mill et al., 2011; Gallagher et al., 2013). In this study increasing herd size has been shown to reduce the time to a subsequent breakdown. This may reflect imperfect specificity of the SICTT (96 – 99 %; Monaghan et al., 1994) or could, as described in Denny and Wilesmith, (1999), be related to the greater risk of contact with contiguous herds or with badgers in the area. De La Rua-Domenach et al. (2006) challenged the idea of poor SICTT specificity, pointing out that the lack of visible lesions in some reactors is not necessarily one of poor SICTT specificity but instead reflects inadequate gold standards to determine true infection standards. This idea has also been investigated in Good et al. (2011) where concerns regarding the specificity of the SICTT of single positive animals in the absence of an obvious source of (bTB) infection were perceived as a “false” positive. Good et al. (2011) however concluded that for the Irish bTB eradication programme data presented was consistent with published literature on the sensitivity, specificity and test reliability of the SICTT. Brooks-Pollock and Keeling (2009) explicitly connect herd size and disease persistence and point out that many elements intrinsic to large herds such as increased number of movements, larger land coverage, increased environmental contamination and more densely stocked cattle are all contributing factors. Brooks-Pollock and Keeling (2009) and Denny and Wilesmith (1999) point to the risk of bTB in large herds as being multifactorial in nature and probably related to increased scale of operations on these types of holdings.

Increased breakdown severity has been shown by this, and other studies (Olea-Popelka et al., 2004; Wolfe et al., 2010; Karolemeas et al., 2011 Gallagher et al., 2013), to be associated with decreased time to subsequent outbreaks. This could be due to spread of the disease within a herd or to the persistence of factors which led to the initial reactors (Olea-Popelka et al., 2004). Karolemas et al. (2011) states that the relative contribution of within-herd persistence and re-introduction of infection to the recurrence of breakdowns is not known. It is however suggested that recurrence after a short time period relates to within herd persistence. All herds in this study had negative breakdown and six-month post-restriction tests which should have acted to reduce the significance of bTB ‘within herd persistence’ though probably not addressing the issue of latently infected animals. This in turn could suggest that the main element driving recurrence of bTB breakdowns in this study is re-introduction of infection to the herds.

Dairy herds were found to be at higher risk of breakdown than non-dairy herds. Dairy herds have been shown to be an additional risk factor in other work (Griffin et al. 1993; Ramírez-Villaescusa et al., 2010; Alvarez et al., 2012). In the initial analysis it was shown that the type of herd was associated with herd size, with dairy herds being larger. This variable however remained significant in the final multivariable model, which included herd size, suggesting that other factors, intrinsic to dairy herds, are associated with the shorter time to breakdown. A possible explanation for this is described in Griffin et al. (1993) where more intensively managed dairy herds were determined to be at a greater risk from tuberculosis than were other herds. Another explanation for increased risk in dairy herds is that dairy cattle reach an older age than cattle intended for meat and so have a longer time to be exposed and infected with bTB (Ramírez-Villaescusa et al., 2010). Alvarez et al. (2012) determined that dairy herds had a higher risk of bTB compared to other herd types, as indicated by larger values of within herd transmission, proportion of outbreaks and proportion of recurrently infected farms. The reasoning given for this finding again relates to management where dairy farms are more likely to have high contact rates, high density and presence of stressors.

Bermingham et al. (2009) investigated genetic susceptibility of the Irish Holstein-Friesian dairy herd. This paper demonstrated that significant genetic variability for susceptibility to confirmed *M. bovis* infection exists. Relative to other cattle in the population the dairy herd has a different genetic background Ramírez-Villaescusa et al. (2010). It is possible that dairy herd genetics could play a role in making this variable significant, though in a Northern Ireland context more work would be required to determine its overall contribution.

One way in which risk of disease could persist is where high bTB prevalence in a locality at the disclosing test is still present when the herd enters the study at its post-outbreak herd test. This possibility was investigated using the variable local prevalence. The results show a threshold level at 12 % prevalence above which the hazard of a future herd breakdown increases as bTB prevalence increases. This may indicate that a certain pivotal concentration of local infection leads to acceleration in number of herd breakdowns. Denny and Wilesmith (1999) argue that the local factors which influence herd breakdown involve a complex interplay of farm boundaries, neighbours and wildlife. The risk presented within a locality by contiguous herds has been identified in previous work (Griffin et al., 1996; Denny and Wilesmith, 1999; Johnston et al., 2011). Associations between infected wildlife and future herd bTB risk have been demonstrated previously (Griffin et al., 1996; Reilly and Courtenay, 2007) In White et al. (2013) the topic of locality is teased apart to determine the relative importance of bTB farm to farm spread and spread from wildlife. It shows that there is a risk from herds directly contiguous and herds >25m from the farm boundary, which is best explained by an infected wildlife source. The variable used in our study (local prevalence) does not attempt to break the locality effect into its components, however Murphy et al. (2011) point to the fact that cattle act as sentinels for TB in badgers. This means cattle prevalence probably acts as a good proxy for local bTB infection pressure, though how it reflects the components of contiguous or wildlife spread is unknown.

Another risk area for bTB is movement of cattle into a herd. This study design meant that herds were present within the study for variable amounts of time and would thus have varying numbers of cattle purchased. To adjust for this, a variable ‘purchase intensity’ was used, which is an integration of these two variables into one measurement - number of animals purchased per unit time. In herds with > 27.38 animals purchased per year there is an increased hazard of bTB breakdown relative to the baseline level of < 0.73 animals purchased per year. The type of herd from which purchases occurred was not taken into account as it could not be accurately ascertained from the original dataset.

Increased risk has been shown to be associated with purchasing cattle from herds with a greater than biennial frequency of testing for bTB in the previous eight years (Carrique-Mas et al., 2008) and purchasing from markets (Johnston et al., 2005; Ramírez-Villaescusa et al., 2010) although some authors have produced work which questions risk associated with cattle movement (Griffin et al., 1996; Abernethy et al., 2000). In Green et al. (2008) British bTB outbreak data for 2004 was used to estimate that 16% of herd infections were directly related to cattle movements. The idea of herd infection initiated by purchase of infected animals is supported by Berrian et al. (2012). In this work the risk of bTB was determined among animals sold out of herds in the Republic of Ireland (ROI) in 2005. Animals sold out of herds with a bTB episode in 2005 before the episode began had more than twice the odds of developing bTB in the subsequent two years compared to those moving out after the episode. It is also a feature of bTB breakdowns where some are more efficient at onward spread of infection than others. This particular aspect is demonstrated in Gopal et al. (2006) where in the study of 31 herds disclosing bTB in north-east England between January 2002 and June 2004 one source herd was linked to nine of the breakdowns.

A Bovine Tuberculosis Biosecurity Study for County Down, Northern Ireland (O'Hagan et al., unpublished) examined purchase of cattle as a risk factor for a bTB breakdown. It was found that the purchase of beef fattening animals in the last 3 years was significantly more common in case farms compared to control farms (adjusted OR=4.60; 95% CI 1.61-13.13; P=0.003). This supports previous research that purchasing potentially infected cattle can be associated with an increased risk of bTB breakdown (O'Hagan et al., unpublished). The majority of cattle herds in Northern Ireland do not have adequate facilities to finish all of their animals, instead making use of livestock markets to trade cattle. When purchasing cattle at a market it is not presently possible to determine the bTB historical status of the vendor's herd. This means the buyer can use no element of risk judgement in the purchase of animals, being equally probable to obtain animals from low or high risk sellers.

The bTB history of a herd has been shown in many studies to be a significant risk factor, where a historical bTB episode makes a future one more probable. (Wilesmith and Williams, 1987; Olea-Popelka et al., 2004; Green and Cornell, 2005; Carrique-Mas et al., 2008, Wolfe et al., 2010, Karolemeas et al., 2011). In this study bTB history was not found to be significantly associated with time to subsequent breakdown and given the chronic nature of bTB this is surprising. Dawson et al. (2012) define the causes of recurrent TB episodes as repeated exposure to infected wildlife vectors, repeated purchase of infected stock and recrudescence of within-herd infection. In Good et al. (2013) full depopulation of high risk bTB herds was compared to depopulation of low risk herds. The risk status given to a herd was based on its bTB history in the previous five years. In the case of the high risk herds a local badger removal policy was implemented. The study determined that there was no substantial difference in future bTB risk between the two groups. Good et al. (2013) thus provides insight into how removal of one of the Dawson et al. (2012) defined causes of recurrent TB episodes can reset the historical status to a lower level. Our study uses a 2 year time period on which to base the bTB history variable, thus categorising herds with a recent breakdown and theoretically at higher risk. Olea-Popelka et al. (2004), found bTB history to significantly increase the hazard of a future bTB breakdown but used a five year period. Carrique-Mas et al. (2008) reported that the reduction in odds of herd breakdown where there was a history of bTB was indistinguishable from an exponential decay distribution. It pointed to the fact that even five years after a herd breakdown there was an OR = 2.9 (95% CI 1.11-7.78) of a further disease incident relative to a herd which did not have a breakdown. A possible explanation as to why bTB history was found not to be significant in this study is the selection process for herds entering the study. The fact that chronically infected herds (herds where disclosure preceded the post-outbreak test by two years or more) and herds with additional risk factors (for example any herd located contiguous to a bTB infected herd) were excluded from the study, may have selected for a type of breakdown in which the historical status was of less importance. Thus given what Dawson et al. (2012) has defined as the causes of recurrent TB episodes, selecting herds without additional risk factors may select those in areas where the wildlife vector component is minimal. Also by including herds with a clear post restriction test the recrudescence factor is minimised as these herds have at that point completed three clear herd tests.

Confirmation of bTB post disclosure was shown not to be significant in the final multivariable model. This is a surprising result given that confirmation has an important status in the Northern Ireland eradication and control programme (Abernethy et al., 2006). Also it could be thought that presence of lesions would indicate a longer standing infection and thus increased risk for a herd. However Abernethy et al. (2010) found that neither confirmation of infection at the disclosure test nor subsequently in the breakdown was a statistically significant risk factor. This same result has been reported in other studies (Olea-Popelka et al., 2004; Wolfe et al., 2010; Karolemeas et al., 2011; Abernethy et al., unpublished).

Conclusion

The original objective of this work was to investigate risk factors affecting the time period from post-outbreak test to a further herd breakdown for cattle herds in Northern Ireland. The key conclusions reached from this study are that increasing herd size, breakdown severity, local bTB prevalence and purchase level above 27.38 animals per year are all associated with a decreased time to subsequent outbreak. The study also concludes that dairy herds are associated with a decreased time to subsequent outbreak. Consistent with other studies this work shows bTB confirmation to be not predictive of a future herd breakdown. However, probably due to the selection processes applied to herds for entry to the study bTB history was found not to be a risk factor for a future herd breakdown.

Conflict of Interest

The authors declare that they are not in a situation of conflicting interests

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Figures and Tables

Figures

Figures 1 to 6: Kaplan-Meier curves calculated on time intervals from post-outbreak test to disease incident and stratified by level of breakdown severity, local prevalence, purchase intensity, herd size, herd type and disease confirmation

Kaplan-Meier curves of time intervals from post-outbreak test to disclosure of bovine tuberculosis in 3377 herds

Figure 1: Stratification by increasing severity of disclosure bTB test.

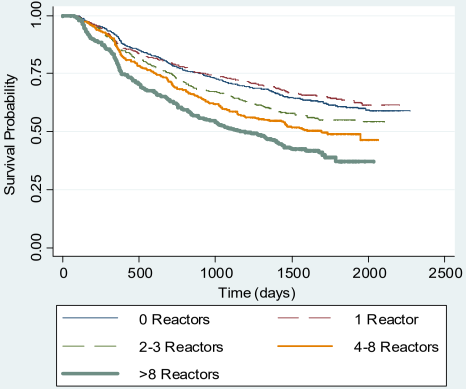

Figure 2: Stratification by increasing levels of local prevalence for bTB.

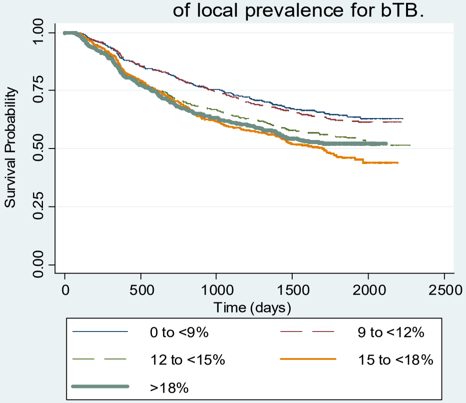

Figure 3: Stratification by increasing levels of purchase intensity (animals/year).

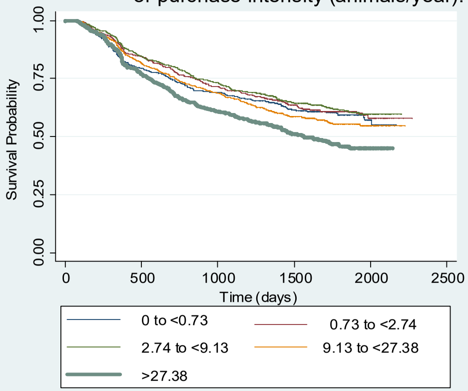

Figure 4: Stratification by increasing herd size.

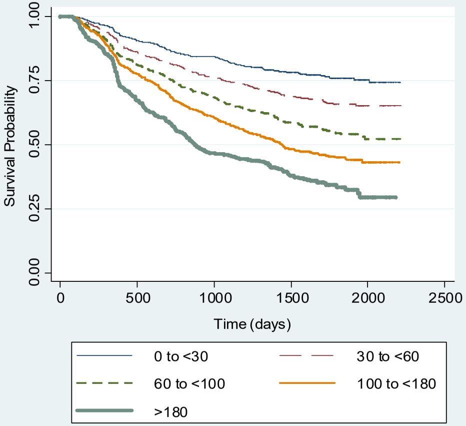

Figure 5: Stratification by herd type: dairy or non- dairy.

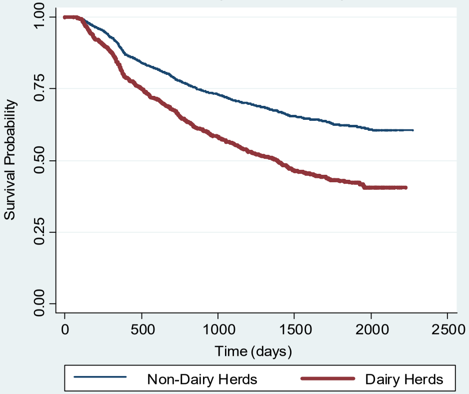

Figure 6: Stratification by bTB confirmation.

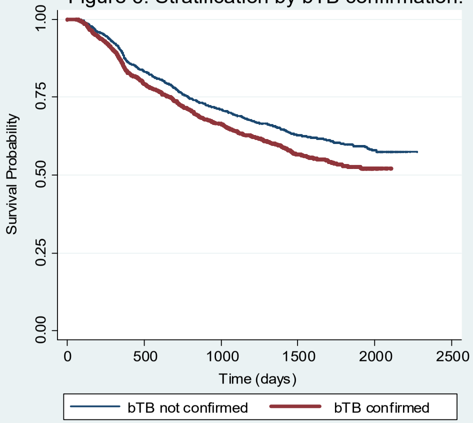

Table 1. Table showing how the 3377 herds with post outbreak tests in Northern Ireland between January 2002 and January 2004 and the 1402 related bTB incidents are distributed across each exposure level of the study variables.

Variable	**Exposure level**	**Number of herds at exposure level**	**Number of herds returning a bTB incident at exposure level**
Herd Size	<30	722	167
	30 - 59	751	244
	60 - 99	738	321
	100 - 179	694	368
	>=180	472	302
Breakdown Severity	0	958	364
	1	956	332
	2 to 3	687	298
	4 to 8	441	216
	>8	335	192
Local Prevalence (%)	<9	591	201
	9 to <12	844	303
	12 to <15	927	404
	15 to <18	550	276
	>18	465	218
Purchase intensity (Animals per year)	<0.73	530	210
	0.73 to <2.74	618	237
	2.74 to <9.13	842	311
	9.13 to <27.38	760	325
	>=27.38	627	319
Herd Type	Non-Dairy	2419	872
	Dairy	958	530

Table 1 continued

Variable	Exposure level	Number of herds at exposure level	Number of herds returning a bTB incident at exposure level
BTB Confirmation	TB not confirmed	1786	698
	TB confirmed	1591	704
TB History	No	2145	862
	Yes	1232	540

Table 2. Univariable Cox regression showing association between selected variables and TB herd breakdowns of 3377 herds in Northern Ireland between January 2002 and January 2004.

Variable	Exposure level	Hazard Ratio	95% C.I. of Hazard Ratio	P-value
Herd Size	<30	1.00	-	P <0.005
	30 - 59	1.50	1.23-1.83	
	60 - 99	2.17	1.80-2.62	
	100 - 179	2.86	2.38-3.44	
	>=180	3.99	3.30-4.82	
Breakdown Severity	0	1.00	-	P <0.005
	1	0.93	0.80-1.08	
	2 to 3	1.25	1.07-1.46	
	4 to 8	1.48	1.25-1.75	
	>8	1.96	1.64-2.33	
Local Prevalence (%)	<9	1.00	-	P <0.005
	9 to <12	1.06	0.88-1.26	
	12 to <15	1.40	1.18-1.66	
	15 to <18	1.65	1.38-1.98	
	>18	1.53	1.26-1.85	

Table 2 continued

Variable	Exposure level	Hazard Ratio	95% C.I. of Hazard Ratio	P-value
Purchase intensity	<0.73	1.00	-	P <0.005
	0.73 to <2.74	0.93	0.77-1.12	
(Animals	2.74 to <9.13	0.89	0.74-1.06	
per year)	9.13 to <27.38	1.07	0.90-1.28	
	>=27.38	1.38	1.16-1.64	
Herd Type	Non-Dairy	1.00	-	P <0.005
	Dairy	1.78	1.60-1.98	
BTB Confirmation	TB not confirmed	1.00	-	P <0.005
	TB confirmed	1.22	1.10-1.36	
TB History	No	1.00	-	P=0.064
	Yes	1.11	0.99-1.23	

Table 3. Table showing multivariable hazard ratios, confidence intervals and significance levels for the described variables in 3377 herds entered into the Cox regression model from 1st of January 2002 to 1st of January 2004.

Variable	**Variable level**	**Hazard Ratio**	**95% C.I. of Hazard Ratio**	**P-Value**
Herd Size	<30	1.00	-	-
	30 - 59	1.44	1.18-1.76	<0.005
	60 - 99	1.91	1.57-2.32	<0.005
	100 - 179	2.43	2.00-2.97	<0.005
	>=180	3.17	2.56-3.92	<0.005
Local Prevalence (%)	<9	1.00	-	-
	9 to <12	1.03	0.86-1.23	0.766
	12 to <15	1.40	1.18-1.67	<0.005
	15 to <18	1.63	1.36-1.96	<0.005
	>18	1.78	1.47-2.17	<0.005
Herd Type	Non-Dairy	1.00	-	-
	Dairy	1.31	1.16-1.49	<0.005
Breakdown Severity	0	1.00	-	-
	1	1.04	0.90-1.21	0.582
	2 to 3	1.19	1.02-1.39	0.025
	4 to 8	1.27	1.07-1.51	0.006
	>8	1.50	1.25-1.79	<0.005
Purchase Intensity	<0.73	1.00	-	-
	0.73 to <2.74	0.88	0.73-1.06	0.170
(Animals	2.74 to <9.13	0.83	0.70-0.99	0.040
per year)	9.13 to <27.38	0.99	0.83-1.18	0.922
	>=27.38	1.23	1.03-1.49	0.025

Chapter 3

Bovine tuberculosis in Northern Ireland: Risk factors associated with duration and recurrence of chronic herd breakdowns

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Bovine Tuberculosis in Northern Ireland: Risk factors associated with duration and recurrence of chronic herd breakdowns

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Abstract

This study investigated 8058 bovine tuberculosis (bTB) confirmed breakdowns occurring in Northern Ireland during the period 2005 to 2010 inclusive. The methodology used two case-control studies; one determined the risk factors associated with long duration bTB breakdowns and the other with recurrent bTB breakdowns. The analyses were implemented using a generalized linear mixed model analysis with variables relating to repeated measures on herds, locality and year of breakdown included as random effects. The case definition for long duration breakdowns (n=679) was any confirmed bTB disclosure with duration greater than one year. The case definition for recurrent breakdowns (n=657) was any confirmed bTB disclosure with duration less than one year, followed by two or more bTB breakdowns within 2 years from the end of the initial bTB breakdown. In the multivariable model based on duration of bTB breakdowns, significant factors were local area bTB prevalence, number of associated cattle herds, total years restricted in the previous five years, total number of bTB reactors during the breakdown and the presence of a bTB lesion at routine slaughter (LRS). The number of bTB reactors at the disclosing test was also significant; with increased numbers associated to reduced odds of a long duration breakdown. In the second analysis based on recurrence of bTB breakdowns, high local area prevalence, movement intensity into the herd, total years restricted in the previous five years, herd size, total number of TB reactors during the restricted breakdown and presence of a LRS were all statistically significant.

Key words: Epidemiology, *Mycobacterium bovis,* Case-Control Study, Chronic, Duration, Recurrence, Cattle.

1.0 Introduction

First attempts at control of bovine tuberculosis (bTB) in Northern Ireland began in 1935 and have been ongoing ever since and despite the present day programme of regular testing, abattoir surveillance and risk testing tuberculosis has remained endemic in the Northern Ireland cattle population (Abernethy et al., 2006; Robinson, 2015). The present cattle population of Northern Ireland is 1.6 million animals residing in approximately 23,000 herds (www.daerani.gov.uk). Farm fragmentation and a business model frequently dependent upon live animal trade has the obvious epidemiological disease consequences of a high degree of herd contact and potential for movement of bTB between farms from traded animals (Abernethy et al., 2006). The Department of Agriculture, Environment and Rural Affairs (DAERA) administers the bTB eradication programme in Northern Ireland, currently at a cost of nearly £30 million per year. During 2014, the annual herd incidence was 6.03% with an annual animal incidence of 0.55%. Abernethy et al. (2006) provided a comprehensive review of the Northern Ireland bTB eradication programme and more detailed statistics on bTB can be found on the DAERA web site (www.daerani.gov.uk).

A feature of bTB which has created much debate is the fact that certain herds seem to have a much greater propensity to develop prolonged and recurrent breakdowns with the term "chronic bTB herd" being applied to this group. During the period from 2001 to 2003, 27% of herds contributed 56% of the total number of bTB reactor animals (DAERA, unpublished data), thus demonstrating that the bulk of Northern Ireland bTB infection appeared in a subset of herds. Analysis of the risk factors associated with chronic bTB breakdowns have been researched in both Great Britain and the Republic of Ireland. These studies investigated how risk factors such as number of bTB reactors (Wolfe et al., 2010; Karolemeas et al., 2011), herd size (Karolemeas et al., 2010; Wolfe et al., 2010; Brooks-Pollock et al., 2013), herd bTB history (Wolfe et al., 2010; Karolemeas et al., 2011), type of herd (Griffin et al., 1993), purchase patterns (Griffin et al., 1993; Reilly and Courtenay, 2007), husbandry practices (Reilly and Courtenay, 2007) and local farm environment (Griffin et al., 1993; Reilly and Courtenay, 2007) impacted upon chronic herd breakdowns. The objective of our study was to investigate risk factors for chronic bTB breakdowns in Northern Ireland, adding to the knowledge already gained from other countries and allowing for better understanding of why some herds continually fall into this chronic category. In turn this could lead to the development of more effective control measures as discussed in the conclusions and policy implications section.

2.0 Materials and methods

2.1 Study population and study design

A study population for this work consisted of 8058 confirmed bTB breakdowns initiating in Northern Ireland during the period 01/01/2005 to 31/12/2010 (N=5960 individual herds). A bTB breakdown was a herd level event, defined as a herd identity in combination with a breakdown start and end date. Breakdown start and end dates define a period of time when a herd loses its bTB free status and also its capacity to trade cattle. By definition a herd could appear multiple times within the dataset as different bTB breakdowns. Confirmation of bTB in any breakdown occurred where any Single Intradermal Comparative Tuberculin Test (SICTT) reactors returned a positive post mortem (where visible lesions consistent with a diagnosis of bTB) or lesion at routine slaughter (LRS) animals obtained a positive histological or bacteriological examination (where histological lesions consistent with a diagnosis of bTB or where *M. bovis* was isolated on culture).

Two case-control studies were carried out. The first of these determined which risk factors were linked to a case based on duration of a bTB breakdown and was termed the bTB duration case-control study. The definition for a case in this study was any bTB breakdown greater than or equal to 365 days in duration, while a control was any bTB breakdown less than or equal to 184 days (median bTB breakdown duration). The second case definition was based on the recurrence of bTB breakdowns, with a case being any bTB breakdown of less than a year in duration followed by at least two further bTB breakdowns within the two following years. A control was defined as any bTB breakdown of less than a year in duration, which did not experience a subsequent bTB breakdown within the following two year period. This second study was termed the bTB recurrence case-control study.

2.2 Data collection and variable definitions

The identification, movement and test history of all individual animals were extracted from the Animal and Public Health Information System (APHIS), which is managed by DARD (Houston, 2001). Explanatory variables defined for the dataset and linked to each bTB breakdown were the year of breakdown start, divisional veterinary office (DVO; n = 10), local area prevalence of bTB in the year before the breakdown, local area prevalence of bTB in the year of the breakdown, herd type, number of other herds epidemiologically linked to the bTB breakdown herd (associated herds), movement intensity into the herd in the 90 days before the start of the bTB breakdown, herd size, number of bTB breakdowns in the previous five years, total time restricted in the previous five years, number of bTB reactors at the disclosing test (the herd level SICTT closest in time to the start of the restriction), total number of bTB reactors during the bTB breakdown and presence of a LRS. The disclosing test was the whole herd SICTT at which bTB was disclosed or, in the case of an LRS or individual SICTT animal, was the herd SICTT which followed it. The year of the start of each bTB breakdown was one of the years from 2005 to 2010. Local area bTB prevalence both in the year before and the year of the bTB breakdown was calculated based on a geographical area called a patch, which is a sub-division of a DVO that comes under the administration of a Veterinary Officer (VO). The local area bTB prevalence was calculated as a percentage of number of bTB breakdowns to total herds SICTT tested in the patch in each of the years (and year before) from 2005 to 2010 inclusive. Thus for each patch the prevalence in the year of the breakdown and the year before was used in the analysis. Figure 1 shows the Northern Ireland DVO patch structure with local area bTB % prevalence for 2010 applied to it. Herd type was either dairy or non-dairy with the former defined as having a milk licence. Within the Northern Ireland agricultural system a particular farm business may have more than one herd identity number linked to it. This could occur where different herds share facilities or more than one family member in the same business has their own herd identity. Such herds are part of the same epidemiological unit and are known as associated herds. Associated herds were allocated to each bTB breakdown as the total number of herd identities linked exclusively to the bTB breakdown herd. Movement intensity was defined as the number of cattle purchased into a herd in the 90 days before a bTB breakdown began and was measured in units of animals per year. Herd size was the number of cattle tested at the first herd level SICTT in the bTB breakdown. The number of bTB breakdowns in the previous five years was an integer value which gave the number of bTB breakdowns linked to a herd in the five years before the start of the present bTB breakdown. Total time restricted links each bTB breakdown to its accumulated total time under bTB restriction in the previous five years. Thus both the previous variables (number of bTB breakdowns and total time restricted) provide historical information about any bTB breakdown, the former providing a variable based on recurrence while the latter is based on duration. The number of bTB reactors at the disclosing SICTT gave an integer value of the number of TB reactors found at the first whole herd SICTT within a bTB breakdown. The total number of TB reactors during a bTB breakdown provided a sum of all TB reactors disclosed during the bTB breakdown. An LRS was defined as any animal not disclosing as an SICTT reactor, but where *M. bovis* was detected on histopathological examination or by bacteriological culture. Presence of an LRS was a binary variable describing the situation where a LRS initiated or was found during a bTB breakdown.

2.3 Data analysis

Microsoft Access^{TM} (Microsoft Corporation, Redmond, WA, USA) and R Version 2.15.0 (The R Foundation for Statistical Computing) were used for all data manipulations and Genstat[1] was used for data analysis. The model framework was a generalised linear mixed model with a binomial distribution and logit link function. The variables of herd identity, local veterinary office (DVO) and year of breakdown were used as random effects within all the models generated. Summary statistics were calculated for each variable and an initial descriptive analysis of the dataset completed to provide background information. The variable movement intensity was categorised to five levels so as to create categories containing approximately equal numbers of observations. A zero level category was included in the categorised variable movement intensity. This zero level category selected breakdowns into which no cattle movement occurred in the 90 days prior to disclosure and represented closed herd management systems. Following the univariable analysis, a multivariable model of the fixed effects was generated by both forward and backward selection of significant variables. A 5% significance level was used for rejection in both the univariable and multivariable models. The other variables within the dataset were used as fixed effects. All two way interactions between variables in the final models were assessed for significance at the 5% level. The final set of terms obtained was an optimised multivariable model for the given case-control study. In order to assess linearity in log odds continuous variables were categorised into categorical variables with 4 levels defined by the median value for each of the four groups. For each variable in turn the continuous variable was replaced by its equivalent categorical variable in the modelling process. The estimated coefficients from each fit (zero being substituted for the reference level) were graphed against each level of the new variable and visually inspected for departures from linearity. Any variable that was not assessed as linear in the logit and that was included in either final multivariable model was fitted with an additional squared term in the model to account for any departure from linearity and its significance tested.

A correlation matrix of all pair wise combination of variables was carried out to assess collinearity and Receiver Operating Characteristic (ROC) curves were plotted for both models. Area Under Curve (AUC) was calculated for both ROC curves to assess the predictive ability of the models.

3.0 Results

In the bTB duration case-control study there were 679 cases and 3145 controls. All the variables included at the univariable level were statistically significant (Table 1). With the duration case definition, the increased odds of

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[1] GenStat for Windows (2013). 16th Edition. VSN International Ltd., Hemel Hempstead, UK

a bTB breakdown per 1% increase in local area prevalence in the year of the breakdown and the year pre the breakdown were OR 1.08 (95% CI: 1.06-1.10) and 1.04 (95% CI: 1.02-1.06) respectively; in dairy herds compared to non-dairy OR 1.43 (95% CI: 1.17-1.74); for each additional associated herd OR 1.61 (95% CI: 1.42-1.82); for every additional ten animal increase in herd size OR 1.03 (95% CI: 1.02-1.03); for each extra bTB breakdown in the previous five years OR 1.40 (95% CI: 1.30-1.50); for each extra year of restriction in the previous 5 years OR 1.82 (95% CI: 1.68-1.97); for every extra SICTT reactor at the disclosing test OR 1.08 (95% CI: 1.06-1.10); for every extra SICTT reactor during the bTB breakdown OR 1.20 (95% CI: 1.17-1.22); and for the presence of an LRS during the bTB breakdown OR 2.23 (95% CI: 1.87-2.66). The variable, movement intensity in the three months prior to the start of restriction, was categorised and only at a purchase level of >100 animals per year compared to no purchases was there an increased odds of a breakdown satisfying the duration case definition; OR 2.02 (95% CI: 1.59-2.56) (Table 1).

In the multivariable model, the variables found to be statistically significant for the duration case definition were local area bTB prevalence in the year of the bTB breakdown; per 1% increase OR 1.06 (95% CI: 1.03-1.10), number of associated herds; per extra associated herd OR 1.35 (95% CI: 1.13-1.61), number of years restricted in the previous five years; per extra restricted year OR 1.35 (95% CI: 1.20-1.52), number of TB reactors during the bTB breakdown; per extra reactor OR 2.15 (95% CI: 1.99-2.33) and presence of a LRS; OR 2.85 (95% CI: 2.18-3.74). The number of TB reactors at the disclosing test was also statistically significant but was associated with reduced odds of developing a long duration bTB breakdown; per extra reactor OR 0.45 (95% CI: 0.40-0.49) (Table 2).

In the bTB recurrence case-control study, there were 657 cases and 4659 controls. All the variables included at the univariable level were statistically significant (Table3). With the recurrence case definition the increased odds of a bTB breakdown per 1% increase in local area prevalence in the year of the breakdown and the year prior to the breakdown were; OR 1.06 (95% CI: 1.04-1.08) and 1.05 (95% CI: 1.03-1.07), respectively, in dairy herds compared to non-dairy; OR 1.36 (95% CI: 1.08-1.73), for each additional associated herd; OR 1.62 (95% CI: 1.40-1.86), for every additional ten animal increase in herd size; OR 1.06 (95% CI: 1.05-1.06), for each additional bTB breakdown in the previous five years; OR 1.78 (95% CI: 1.65-1.92), for each extra year of restriction in the previous 5 years; OR 2.68 (95% CI: 2.43-2.94), for every additional SICTT reactor at the disclosing test; OR 1.03 (95% CI: 1.02-1.05), for every additional extra SICTT reactor during the bTB breakdown; OR 1.03 (95% CI: 1.02-1.04), and for the presence of an LRS during the bTB breakdown; OR 2.18 (95% CI: 1.79-2.66). The variable, movement intensity in the three months prior to the start of restriction, was categorised and only at the two most extreme purchase levels (>25-100 and >100 animals per year) compared to no purchases was there an increased odds of a breakdown satisfying the recurrence case definition; OR 1.62 (95% CI: 1.19-2.20) and OR 7.92 (95% CI: 6.05-10.37), respectively (Table 3). With the multivariable model, the variables found to be statistically significant for the recurrence case definition were local area bTB herd prevalence in the year of the bTB breakdown; per 1% increase OR 1.05 (95% CI: 1.03-1.08), movement intensity into the herd in the 90 days prior to the bTB breakdown; >100 animals per year OR 4.24 (95% CI: 3.15-5.71), the number of years restricted in the previous five years; per extra restricted year OR 1.93 (95% CI: 1.73-2.14), herd size; per extra ten animals OR 1.03 (95% CI: 1.02-1.04), number of TB reactors during the bTB breakdown; per extra reactor OR 1.02 (95% CI: 1.01-1.04) and the presence of a LRS; OR 1.43 (CI: 1.14-1.78) (Table 4).

None of the interaction terms significant at the 5% level were deemed to be biologically plausible and therefore omitted from the final model. Assessment of the continuous variables for linearity of log odds determined that there were no significant departures from linearity for any variables included in the final multivariable models.

Results from correlation assessment between variables to assess collinearity showed there to be no pair wise combination of variables demonstrating significant correlation. The bTB duration model gave an AUC of its ROC curve of 0.930 while the recurrence model had an AUC of 0.942. The values given for the AUC's of the study models show that they provided a very good accuracy of prediction.

4.0 Discussion

Within Northern Ireland, this is the first time that duration and recurrence has been used to investigate the risk factors for chronic bTB herd breakdowns. Although similar studies have been carried out in other countries (Reilly and Courtenay, 2007; Brooks-Pollock and Keeling, 2009; Karolemeas et al., 2010, 2011) differences in demography, farming practices and geographical location do not necessarily allow extrapolation of their findings to the Northern Ireland situation. Moreover, this investigation of chronic bTB herd breakdowns was commissioned by the government Department's bTB Programme Steering Group and also recommended within the Northern Ireland Assembly's Agricultural committee's review of bTB (Anonymous, 2012). Our bTB duration case-control study used a definition of greater than one year for cases. Other studies have used periods of twice the minimum restriction period for a confirmed bTB breakdown (>240 days; Karolemeas et al. (2010), greater than six months Reilly and Courtenay (2007) or the same definition (>365 days) as used in this study Griffin et al. (1993)). Arguably the longer duration (> 365 days) was more appropriate as it incorporated the bTB breakdowns of more extreme durations. Indeed, each case-control study (duration and recurrence) independently included approximately 8% of the total number of bTB breakdowns from the study population.

From both our case-control studies immediate local bTB prevalence was identified as a contributing factor for both prolonged duration and recurrent breakdowns. This supports the findings of White et al. (2013) where 'neighbourhood' was related to the persistence of bTB in Irish cattle herds. In such highly infected localities, rapid deployment of control measures may act to mitigate development of chronic bTB breakdowns.

Increased number of SICTT reactors at the disclosing test was found to be significantly associated with reduced odds of developing a prolonged duration breakdown. One possible explanation for this would be that the more severe an initial intervention the more likely it is that herd infection is cleared. It is also probable that in larger outbreaks more negative-in-contact animals (animals which can be epidemiologically linked to reactor animals but which were negative SICTT) are removed, further reducing the infection load within a herd.

Increasing number of SICTT reactors disclosed during a bTB breakdown were statistically significant in their association to long duration and recurrent bTB breakdowns, which is consistent with findings elsewhere (Olea-Popelka et al., 2004; Wolfe et al., 2010; Karolemeas et al., 2011; Gallagher et al., 2013). However, this variable may be confounded by the response variable in the bTB duration study, where increasing numbers of reactors were a prerequisite for increased breakdown duration (see Karolemeas et al. (2010)).

The disclosing of each additional reactor over the period of a restriction increased the odds of a future recurrent breakdown pattern; OR 1.02 (95% CI: 1.01-1.04). This suggests that at least some herds were being derestricted prematurely. Alternatively, the increased total numbers of reactors was reflective of ongoing re-infection of the herd, including cattle or wildlife reservoirs, leading to reduced probability of clearance and thus increased likelihood of recurrence. Wildlife variables were not included in this study as data of sufficient quality and geographical coverage for the period of time of the analysis were not available.

Movement of cattle into a herd was only a risk factor in high volume movements with recurrent breakdowns, which appears contrary to the findings in a GB study (Karolemeas et al., 2010). However, our study monitored purchase behaviour before the breakdown began, as opposed to during the bTB breakdown, when purchasing behaviour is likely to be disrupted from normal patterns. A period 90 days pre breakdown was chosen to monitor cattle movement into a herd as background information determined this to be a good representation for purchase patterns of a herd. With recurrent breakdowns movement was only significant at the most extreme level where herds were purchasing more than 100 animals per year. Using a similar metric, Reilly and Courtenay (2007) found that purchase of more than 50 animals was a risk factor for transient bTB restriction (defined as being less than six months). It must also be noted that in our work the case and control breakdowns were all bTB infected breakdowns. This means the results broadly confirm movement into a herd as a risk factor for recurrent breakdowns.

A herd history of bTB has been described as a risk factor for future outbreaks (Olea-Popelka et al., 2004; Carrique-Mas et al., 2008; Gallagher et al., 2013; White et al., 2013). Our study showed that both long duration and recurrent breakdowns were best explained by the total time a herd was restricted in the previous five years. Thus the degree to which there is historical sustenance of infection may provide an indicator towards herds where the environment, including wildlife reservoirs favours the maintenance of disease.

In our study herd size has been shown to be a risk factor for recurrent bTB breakdowns but not long duration breakdowns. This positive association found between herd size and recurrence was consistent with previous studies (Olea-Popelka et al., 2004; Abernethy et al., 2010; Wolfe et al., 2010) but inconsistent with Karolemeas et al. (2011). Also Reilly and Courtenay (2007) showed that both transient and prolonged breakdowns had increased odds with increased herd size. With long duration breakdowns in a herd, maintenance of the infection or constant re-infection is necessary. However with recurrent cases, at least two clear herd tests must be completed before derestriction. Placed within the context of herd size, this may indicate that when there is increased infection pressure, the size of the herd is not important; its only significance relates to lower infection pressures where large herd breakdowns tend to recur at a greater rate. It should however be borne in mind that lack of statistical significance for the association between herd size and long duration breakdowns may not necessarily relate to biological importance.

The presence of a LRS was found to be associated with both long duration and recurrent breakdowns. It is possible that management realignments occur in herds subject to chronic bTB breakdowns where trading restrictions may result in more cattle going directly to slaughter and therefore disclosing more infection through this route.

5.0 Conclusions and policy implications

Present control strategies implement protocols of contiguous testing around bTB confirmed breakdowns. However, this approach in heavily infected localities may not be enough to prevent herds in these areas developing chronic bTB characteristics. Given that the case definitions used in our study encompass almost 40% of the total number of SICTT reactors identified during the study period, focusing disease control resources towards such herds could be potentially very beneficial. The negative association demonstrated between number of SICTT reactors at disclosure and odds of the breakdown attaining a long duration merits further investigation. This suggests that more aggressive initial intervention in such cases (for instance application of severe interpretation and removal of negative in contact animals) would lead to more favourable long term outcomes. However, further work may be required to develop policy in this area. Along similar lines, we observed that the odds of a recurrent breakdown were increased with total reactor numbers found during a bTB breakdown of less than one year. This may indicate that some herds with multiple bTB reactors were not restricted for a long enough duration and more stringent policies need to be applied to ensure they are cleared of infection before derestriction. Options may include more sustained use of severe interpretation and parallel testing with supplemental tests such as interferon-gamma testing. On reflection, one area that would complement the study is future assimilation of more farm level management data. The additional source of more field data would complement the current models that are based purely on data available from a centralised database; thus providing further insights into the epidemiology of bTB in Northern Ireland.

6.0 Acknowledgements

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7.0 Conflict of interest

The authors declare that they are not in a situation of conflicting interests.

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9.0 Tables and Figure

Table 1: Duration case-control study with each of the explanatory variables used as a single fixed level term. Each single variable fixed effect model contained herd identity, local veterinary office (DVO) and year of breakdown as random effects. All listed variable P-Values<0.05.

Variable	**Exposure level**	**Odds Ratio**	**95% Confidence Interval**	**Categorised Variables: Number of Case/Controls per Level**		**Non-Categorised Variables: Median (Range 5-95%)**	
				Cases	**Controls**	**Cases**	**Controls**
Local area prevalence: Year of breakdown (%)	Per 1% increase	1.08	1.06-1.10	679	3145	7.72 (3.33-19.27)	6.71 (2.54-17.27)
Local area prevalence: Year pre breakdown (%)	Per 1% increase	1.04	1.02-1.06	679	3145	7.71 (2.46-18.67)	6.58 (1.99-17.05)
Herd Type	Non-Dairy	-	-	485	2445	-	-
	Dairy	1.43	1.17-1.74	194	700	-	-
Number of associated herds	Per extra associated	1.61	1.42-1.82	679	3145	0 (0-1)	0 (0-2)
Movement intensity into herd in the three months prior to restriction start (animals/year)	0	-	-	253	1325	-	-
	>0 to 10	1.01	0.78-1.31	101	537	-	-
	>10 to 25	0.96	0.70-1.30	63	356	-	-
	>25 to 100	1.04	0.80-1.36	100	488	-	-
	>100	2.02	1.59-2.56	162	439	-	-
Herd size	Per ten extra animals	1.03	1.02-1.03	679	3145	126 (17-525)	74 (11-352)
Number of bTB breakdowns in the previous five years	Per extra bTB breakdown	1.40	1.30-1.50	679	3145	1 (0-4)	1 (0-3)
Number of years restricted in the previous five years	Per extra restricted year	1.82	1.68-1.97	679	3145	0.93 (0.00-4.00)	0.28 (0.00-2.31)
Number SICTT reactors at the disclosing test	Per extra reactor	1.08	1.06-1.10	679	3145	1 (0-18)	1 (0-7)
Total number of SICTT reactors during the bTB breakdown	Per extra reactor	1.20	1.17-1.22	679	3145	8 (0-72)	1 (0-8)
Lesion at routine slaughter during bTB breakdown	No	-	-	342	2228	-	-
	Yes	2.23	1.87-2.66	337	917	-	-

Table 2: Multivariable model generated on the duration case-control study. Herd identity, local veterinary office (DVO) and year of breakdown have been included as random effects. All listed variable P-Values<0.05. (Cases/Controls N=679/3145). Random variables estimated variance components: DVO = 0.26, DVO.HerdIdentity = 0.00, DVO.HerdIdentity.YearOfBreakdown = 0.00.

Variable	**Exposure level**	**Odds Ratio**	**95% Confidence Interval**
Local area prevalence: Year of breakdown (%)	Per 1% increase	1.06	1.03-1.10
Number of associated herds	Per extra associated herd	1.35	1.13-1.61
Number of years restricted in the previous five years	Per extra restricted year	1.35	1.20-1.52
Number of SICTT reactors at the disclosing test	Per extra reactor	0.45	0.40-0.49
Total number of SICTT reactors during the bTB breakdown	Per extra reactor	2.15	1.99-2.33
Lesion at routine slaughter during bTB breakdown	No	-	-
	Yes	2.85	2.18-3.74

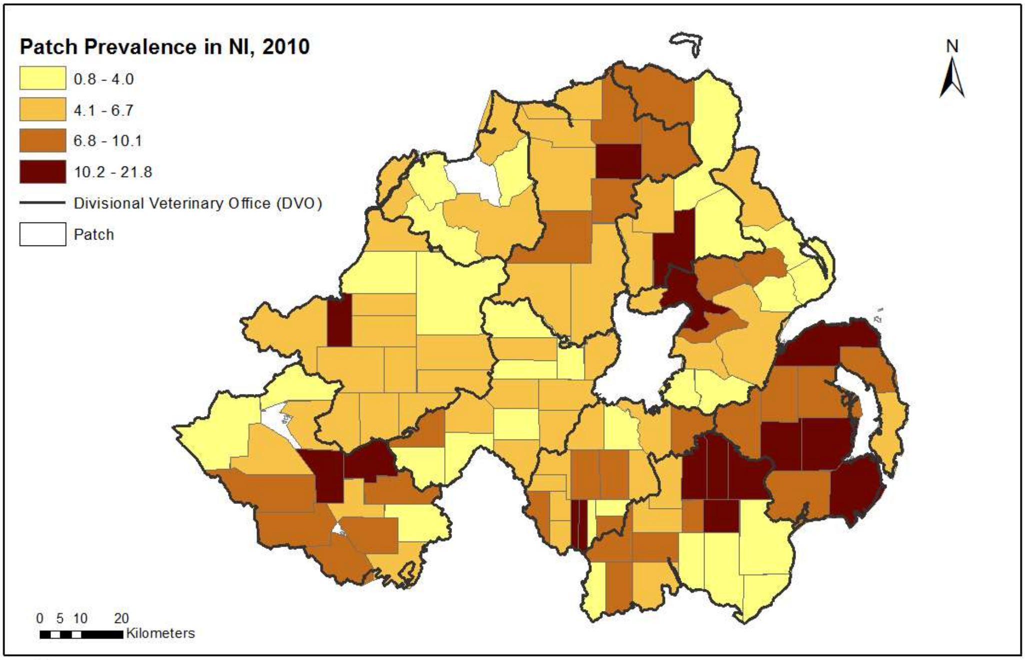
Table 3: Recurrence case-control study with each of the explanatory variables used as a single fixed level term. Each single variable fixed effect model contained herd identity, local veterinary office (DVO) and year of breakdown as random effects. All listed variable P-Values<0.05.

Variable	**Exposure level**	**Odds Ratio**	**95% Confidence Interval**	**Categorised Variables: Number of Case/Controls per Level**		**Non-Categorised Variables: Median (Range 5-95%)**	
				Cases	**Controls**	**Cases**	**Controls**
Local area prevalence: Year of breakdown (%)	Per 1% increase	1.06	1.04-1.08	657	4659	7.87 (2.93-23.30)	6.74 (2.54-17.26)
Local area prevalence: Year pre breakdown (%)	Per 1% increase	1.05	1.03-1.07	657	4659	7.47 (2.45-23.58)	6.57 (1.96-14.91)
Herd Type	Non-Dairy	-	-	505	3719	-	-
	Dairy	1.36	1.08-1.73	152	940	-	-
Number of associated herds	Per extra associated	1.62	1.40-1.86	657	4659	0 (0-2)	0 (0-1)
Movement intensity into herd in the three months prior to restriction start **(animals/year)**	0	-	-	144	2040	-	-
	>0 to 10	1.38	1.01-1.88	80	839	-	-
	>10 to 25	1.10	0.75-1.63	42	536	-	-
	>25 to 100	1.62	1.19-2.20	90	751	-	-
	>100	7.92	6.05-10.37	301	493	-	-
Herd size	Per ten extra animals	1.06	1.05-1.06	657	4659	198 (23-721)	69 (10-307)
Number of bTB breakdowns in the previous five years	Per extra bTB breakdown	1.78	1.65-1.92	657	4659	2 (0-5)	1 (0-3)
Number of years restricted in the previous five years	Per extra restricted year	2.68	2.43-2.94	657	4659	1.58 (0.00-4.19)	0.23 (0.00-2.10)
Number SICTT reactors at the disclosing test	Per extra reactor	1.03	1.02-1.05	657	4659	1 (0-15)	1 (0-9)
Total number of SICTT reactors during the bTB breakdown	Per extra reactor	1.03	1.02-1.04	657	4659	2 (0-22)	1 (0-13)
Lesion at routine slaughter during bTB breakdown	No	-	-	299	3282	-	-
	Yes	2.18	1.79-2.66	358	1377	-	-

Table 4: Multivariable model generated on the recurrence case-control study. Herd identity, local veterinary office (DVO) and year of breakdown have been included as random effects. All listed variable P-Values<0.05. (Cases/Controls N=657/4659). Random variables estimated variance components: DVO = 0.11, DVO.HerdIdentity = 0.91, DVO.HerdIdentity.YearOfBreakdown = 0.00.

Variable	Exposure level	Odds Ratio	95% Confidence Interval
Local area prevalence: Year of breakdown (%)	Per 1% increase	1.05	1.03-1.08
Movement intensity into herd in the three months prior to restriction start (animals/year)	0	-	-
	>0 to 10	1.35	0.98-1.87
	>10 to 25	0.97	0.64-1.47
	>25 to 100	1.34	0.97-1.87
	>100	4.24	3.15-5.71
Number of years restricted in the previous five years	Per extra restricted year	1.93	1.73-2.14
Herd size	Per extra ten animals	1.03	1.02-1.04
Total number of SICTT reactors during the bTB breakdown	Per extra reactor	1.02	1.01-1.04
Lesion at routine slaughter during bTB breakdown	No	-	-
	Yes	1.43	1.14-1.78

Figure 1: Northern Ireland DVO patch structure with local area % bTB prevalence in 2010.



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DARD

Chapter 4

Bovine tuberculosis in Northern Ireland: quantification of the population disease-level effect from cattle leaving herds detected as a source of infection

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Original Paper

Bovine Tuberculosis in Northern Ireland: Quantification of the population disease level effect from cattle leaving herds detected as a source of infection

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Running head: Bovine tuberculosis surveillance

SUMMARY

Determination of the proportion of bTB (bovine tuberculosis) breakdowns attributed to a herd purchasing infected animals has not been previously quantified using data from the Animal and Public Health Information System (APHIS) database in Northern Ireland. We used a case-control study design to account for the infection process occurring in the disclosing bTB breakdown herds. Cases (N=6926) were cattle moving to a future confirmed bTB breakdown where they would disclose as a confirmed bTB reactor or LRS (Lesion at Routine Slaughter). Controls (N=303499) were cattle moving to a future confirmed bTB breakdown where they did not become a bTB reactor or LRS. Our study showed that cattle leaving herds which disclosed bTB within 450 days had an increased odds of becoming a confirmed bTB reactor or LRS compared to cattle which left herds that remained free for 450 days (OR= 2.09: 95% CI: 1.96-2.22). Of the 12060 confirmed bTB breakdowns included in our study (2007-2015 inclusive), 31% (95% CI: 29.8-31.5) contained a confirmed bTB reactor(s) or LRS(s) at the disclosing test which entered the herd within the previous 450 days. After controlling for the infection process occurring in the disclosing bTB breakdown herd, our study showed that 6.4% (95% CI: 5.9-6.8) of bTB breakdowns in Northern Ireland were directly attributable to movement of infected animals.

Key words: Epidemiology, *Mycobacterium bovis,* Case-Control Study, Movement, Cattle.

INTRODUCTION

Tuberculosis is an infectious disease that affects practically all vertebrates and is caused by acid-fast bacilli of the genus *Mycobacterium.* Classically it is defined as a chronic debilitating disease; however it occasionally assumes an acute and rapidly progressive course. Three main types of tubercle bacilli are recognised: human, bovine and avian with *Mycobacterium bovis* being the common cause of tuberculosis in cattle [1].

In Northern Ireland, first attempts at control of bovine tuberculosis (bTB) began in 1935 (compulsory since 1959) and despite the current programme of regular testing and abattoir surveillance, tuberculosis has remained endemic in the Northern Ireland cattle population (1.6 million animals). Within this population there are approximately 285000 dairy cows and 279000 beef cows residing in approximately 23000 herds with average size of 64 animals [2]. During 2015, the annual herd incidence was 7.15% (equating to 2095 herds with bTB reactors) with an annual animal incidence of 0.66% (equating to 11002 bTB reactor animals). Abernethy et al. [3] provided a comprehensive review of the Northern Ireland bTB eradication programme and more detailed statistics on bTB can be found on the DAERA (Department of Agriculture, Environment and Rural Affairs) web site [4].

Movement of infected cattle between herds is considered an important route of dissemination of bTB. The proportion of bTB herd breakdowns attributed to the purchase of infected cattle has been estimated in Ireland (6-7%) and Great Britain (16%) [5, 6]. In Northern Ireland, although purchase of beef/store animals from herds that had a bTB breakdown in the last three years was shown to be a risk factor for a bTB breakdown, no quantification of the effect at a national level was calculated [7]. Other studies that have investigated cattle movement as a risk factor for bTB breakdowns have looked at different aspects of this complicated issue [5, 6, 8-12]. Risk factors that have been demonstrated include cattle movements from markets and farm sales [8, 12], cattle purchased from herds with a higher risk of having bTB [10] and the severity of the original bTB breakdown [11].

Purchased cattle can either be infected with bTB prior to joining the recipient herd or become infected during their residency [13]. Previous studies have not taken the latter post-movement exposure into account within their study design [5, 6, 8-13]. The case-control study presented in this paper was designed to overcome this issue by ensuring that cases and controls were exposed to the same temporal and environmental conditions and hence negating against effects while resident in the recipient herd.

This study used the Northern Ireland bTB surveillance programme to investigate the contribution of cattle movement to overall bTB infection levels.

Study Objective

The objective of this case-control study was to quantify the likelihood that movement of bTB infected animals were responsible for future bTB confirmed herd breakdowns and to determine their impact at a population level.

MATERIALS AND METHODS

Study population and study design

The study population consisted of cattle moving to recipient herds, which would become confirmed bTB breakdowns, with explanatory variables based on their previous donor herds. A confirmed bTB breakdown was defined as a herd with at least one confirmed bTB reactor or a herd with an animal at routine slaughter that was confirmed as having bTB through histological or bacteriological examination (LRS). A confirmed bTB reactor was defined as an animal that had been interpreted as having a positive result to the single intradermal comparative tuberculin test (SICTT) and was also found to have bTB type lesions at post-mortem examination and/or was diagnosed as bTB through histological or bacteriological examination. The disclosure date of a confirmed bTB breakdown was the date of a SICTT at which a confirmed bTB reactor was found or the SICTT herd test date after disclosure of the LRS.

The cattle traced to confirmed bTB breakdowns formed the cases and controls for the study. Cases (N=6926) were traced cattle that had entered a herd that would become a confirmed bTB breakdown where they would disclose as a confirmed bTB reactor or LRS. Controls (N=303499) were cattle that had entered a herd that would become a confirmed bTB breakdown but did not disclose as a confirmed bTB reactor or LRS.

The cattle movements eligible for inclusion in the study occurred in the period 01 January 2007 to 01 August 2014. This time period allowed for a minimum of 450 days follow up of the donor herds after the departure of the case/control animals (e.g. from 1 August 2014 to 1 January 2016).

In this study, the risk window for each bTB herd breakdown was initiated on the date of receipt of the first case animal and finished on the date the herd became a confirmed bTB breakdown. This risk window was used to identify cases and controls that joined the recipient herd. The risk window was limited to a maximum duration of 450 days (15 months). The value of 450 days was used as it is an absolute limit placed on inter herd test intervals within the Northern Ireland bTB eradication programme.

For each of the cases and controls, all herds in which they had been resident during the 450 days prior to their associated risk window disclosure date were identified. Any herd supplying both case and control animals was excluded from the analysis. Explanatory variables for this study were based on the herds in which study animals were resident in prior to moving to their respective risk windows.

The design compared the number of bTB breakdowns in herds through which case animals had resided to the number of bTB breakdowns in herds through which control animals had resided (Figure 1). Thus whether the herds supplying animals into risk windows have a bTB breakdown within a certain time period provides information on the previous environment of the case or control animals. Cases were resident in one or more herds, before arrival in the bTB breakdown herd and either entered into the risk window already infected or became infected in the bTB breakdown herd. If a higher proportion of animals became infected within the trace herds compared to controls then the rate of bTB breakdown in herds supplying cases will be greater than those supplying controls. Such a finding would indicate the possible need to enhance the bTB eradication programme measures in mitigation against this risk to recipient herds.

Data collection and variable definitions

The identification, movement and test history of all individual animals were extracted from the Animal and Public Health Information System (APHIS), which is managed by DAERA [14].

Explanatory variables defined for the dataset and linked to the herds supplying study animals where disclosure of a bTB breakdown less than 450 days after the study animal had left the herd and number of days to the closest bTB herd test after the animal movement. Other explanatory variables include local area bTB incidence in the year of the animal movement, herd size, movement intensity into the herd in the 90 days prior to the animal movement, herd type, bTB herd history and divisional veterinary office (DVO; n = 10). Disclosure of a bTB breakdown less than 450 days after the animal had left a herd was included as a binary variable. Number of days to the closest bTB herd test after the animal movement was allocated a unit of per 30 days, thus representing a monthly effect. This variable was included to account for the fact that within the Northern Ireland bTB testing programme herds moving case animals will be more likely to have a tracing test imposed compared to herds moving control animals. Local area bTB incidence in the year of animal movement from a herd was calculated based on a geographical area called a patch, which is a sub-division of a DVO that comes under the administration of a Veterinary Officer (VO). The local area bTB incidence was calculated as the number of new bTB breakdowns (breakdowns could be initiated by bTB reactor disclosure at or confirmed LRS) to total herds bTB tested in the patch in each of the study years and expressed as a percentage. Thus for each patch, the bTB incidence in the year of study animal movement from a herd was used in the analysis. Herd size was determined as the number of cattle tested in the trace herd at the first bTB herd test after movement of the study animal. Movement intensity was defined as the number of cattle purchased into a herd in the 90 days before the study animal left the trace herd and was measured in units of animals per year. Movement intensity was categorised to five levels so as to create a zero category for closed herds and four other levels containing approximately equal numbers of observations. The zero level category selected situations into which no cattle movement occurred in the 90 days prior to the study animal leaving a herd and represented closed herd management systems. Herd type was either dairy or non-dairy with the former defined as having a milk licence and this was included in the study as a binary variable. BTB herd history was defined as the presence of a bTB breakdown (presence of bTB reactor(s) or LRS(s)) during the two year period prior to the movement of a study animal from the herd and was included in the study as a binary variable. DVO was a naturally categorised variable representing the ten local veterinary administrative areas.

Data analysis

Microsoft Access^{TM} (Microsoft Corporation, Redmond, WA, USA) and R Version 2.15.0 (The R Foundation for Statistical Computing) were used for all data manipulations and StataIC 12[2] was used for data analysis. Summary statistics were calculated for each variable and an initial descriptive analysis of the dataset completed to provide background information. The model framework used in this study was binary logistic regression using clustered robust standard errors. The response variable was animals disclosing with bTB (cases) or not disclosing (controls) after moving from donor herds to the risk window of their recipient herd. Explanatory variables for the analysis were as previously described. The cluster term used in the analysis was the identifier (official tag number) for each study. Use of this identifier grouped the herds through which a study animal moved on their way to a recipient herd. For example, if an animal moved through two herds prior to it entering the recipient herd its identifier linked these two herds into the cluster term.

The variables number of days to the closest bTB herd test, local area bTB incidence and herd size were assessed for their inclusion in the analysis as continuous, without categorisation. This was initially carried out visually by inspecting their lowess (locally weighted scatter plot smoothing) curves with a superimposed linear predictor. Any of the linear predictors which showed departures from the lowess curves were modelled in more detail by spline regression to determine if piecewise representations provided better outcome predictors. If a piecewise representation could be shown to provide a better model than the full linear predictor for any of the continuous variables then the variable was categorised at the cut off points [15].

An initial univariable analysis was applied to each variable to determine if they were statistically significant, at the P=0.2 level. As all variables initially chosen for the study were considered important potential risk factors they were not immediately removed on the basis of the univariable analysis result. Instead a full multivariable model with all study variables was fitted so that any variable non-significant in the univariable analysis at the P=0.2 level and non-significant at the P=0.05 level in the multivariable model would be removed. The variables selected by this process formed the new full model. In the new full model, each of the explanatory variables was in turn individually removed to produce a series of reduced models; each containing one explanatory variable less than the full model. Each reduced model was compared for fit to the full model using BIC (Bayesian information criterion) comparisons. If a reduced model provided a better fit than the full model (that is if the reduced model had a lower BIC value) then the specified explanatory variable was removed from the analysis [16].

All biologically plausible two way interactions were assessed to determine if their addition improved overall model fit. Any interaction which improved the model fit by lowering of the BIC value was selected for inclusion in the final model [16]. A final multivariable model including selected interactions was compared to the original full model, without interactions to determine if their inclusion improved overall fit. A correlation matrix of all pair wise combinations of variables assessed using Pearson product-moment correlation coefficients

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[2] StataCorp. 2011. *Stata Statistical Software: Release 12*. College Station, TX: StataCorp LP.

implemented in STATA^{TM} was carried out to assess collinearity. The finalised model was subjected to a Hosmer-Lemeshow goodness of fit test (decile sub grouped) to determine how well it fitted the data.

We calculated the attributable fraction (AF_e) (adjusted for interaction terms) of bTB infection in individuals exposed to the risk factor of leaving herds which disclosed bTB reactors or LRSs within 450 days [17]. Also calculated was the population attributable fraction (PAF) for diseased animals (cattle disclosing as bTB reactors or LRSs in confirmed bTB breakdowns) attributable to the risk factor of them leaving herds which had a breakdown within 450 days which in turn allowed calculation of the number of cases attributed to it [17]. As these cases were either cattle disclosing as bTB reactors or LRSs in confirmed bTB breakdowns, if each animal went individually to a different recipient herd they would have the potential to generate the same number of breakdowns. However any group of purchased cattle will disperse to recipient herds as singles, doubles, triples etc. meaning they will move to fewer herds than the total number of animals. To account for this effect, we applied the movement distribution for all cases to the subgroup selected as the PAF to determine the number of bTB breakdowns during the study period which received diseased animals.

RESULTS

In this case-control study, there were 6926 cases and 303499 controls drawn from all the Northern Ireland bTB testing and movement data in the study 2007 to 2015 inclusive. During this period, there were 12060 bTB confirmed breakdowns, of which 3695 (31%) had risk windows with case animals. Removal of risk windows that included herds which supplied both case and control animals left 2958 (24%) eligible risk windows. Of the remaining breakdowns 6773 (56%) had only control animals purchased during the 450 day period prior to breakdown initiation while 1592 (13%) had not purchased any animals during the risk window.

Of the three continuous variables assessed for categorisation (number of days to the closest bTB herd test, local area bTB incidence and herd size) only herd size was categorised. Herd size was categorised at the levels 0-50, 51-100, 101-150, 151-300 and >300 as piecewise representation using spline regression at these cut off points provided a better fit of the data than the continuous representation of the variable.In the univariable analysis, statistically significant variables ($P<0.2$) were bTB herd disclosure within 450 days after the animal left the herd, number of days to closest bTB herd test, local area bTB incidence, herd size, movement intensity into the herd and local DVO of the case or control animal prior to move out. The variables herd type and bTB herd histories were not statistically significant. (Table 1).

In the multivariable analysis, all eight explanatory variables were statistically significant ($P<0.05$); thus satisfying the selection criteria to be included in the full model. As a result of model fitting the variables retained in the final model were bTB herd disclosure within 450 days after the animal left the herd, number of days to closest bTB herd test, local area bTB incidence, herd size, movement intensity, bTB herd history and DVO of the donor herd. Herd type was not included in the final model as its presence in the full model did not improve the fit over a reduced model. BTB herd history was not statistically significant in the univariable analysis but statistically significant in the multivariable analysis and its addition to the model was shown to increase overall model fit. As bTB herd history fulfilled the selection criteria it was retained as part of the final model.

Included in the final multivariable model (Table 2) was a two-way interaction term acting between the variables bTB herd disclosure within 450 days after the animal left the herd and time period to closest bTB herd test after the animal move. This was the only interaction which improved model fit. Inclusion of the interaction term meant that assessment of the odds ratio for the variable bTB herd disclosure within 450 days after animal movement required interpretation based on the time period to the closest bTB herd test (Figure 2).

For a time period to the closest bTB test of 111 days (median of the variable; IQR: 56-189), cattle leaving herds which disclosed bTB within 450 days compared to cattle that left herds that did not disclose bTB were associated with an odds of becoming a bTB reactor or LRS of 2.09 (95% CI: 1.96-2.22) and this odds decreased with increasing time since closest bTB test. Also, for every 1% increase in local area bTB incidence, the odds of an animal becoming a bTB reactor or LRS in the recipient herd increased (OR= 1.02: 95% CI 1.01-1.03). Regarding herd size, the odds of an animal becoming a bTB reactor or LRS was lower in all the larger herd size categories compared to the base level category of 0-50 cattle (Table 2). Increased purchasing (movement) intensity showed increased odds of an animal leaving the herd becoming a bTB reactor or LRS. At a purchase intensity of >100 animals per year in the three months prior to an animal leaving the herd, the odds of it becoming a bTB reactor or LRS increased compared to animals leaving herds which did not purchase animals (OR= 1.46: 95% CI 1.37-1.59). With herds that had a bTB breakdown in the two years prior to an animal movement, there was a decreased odds of becoming a bTB reactor or LRS compared to cattle leaving herds with no bTB (OR= 0.85: 95% CI 0.80-0.92). Animals moving from herds in DVO Enniskillen, DVO Newry and DVO Newtownards (DVO5, DVO8 and DVO9) relative to DVO Armagh (DVO1) had the greatest increase in odds of becoming a bTB reactor or LRS (OR= 1.35: 95% CI: 1.21-1.51, OR= 1.27: 95% CI: 1.14-1.43 and OR= 1.35: 95% CI: 1.18-1.53, respectively).

Collinearity assessment between variables showed there to be no linear correlations between variables with a correlation coefficient in excess of 0.47, which was recorded between the variables herd type and herd size. The Hosmer-Lemeshow goodness of fit test indicated a reasonable fit for the full multivariable model (P = 0.42).

The AF_e in individuals exposed to the risk factor of leaving herds which disclosed bTB reactors or LRSs within 450 days was determined using the odds ratio returned in the multivariable model adjusted for the interaction term; (OR=2.09: 95% CI: 1.96-2.22; $AF_e = 0.52$: 95% CI: 0.49-0.55). Thus 52% of the bTB infection in individuals leaving herds which disclosed bTB reactors or LRSs within 450 days was accounted for by this exposure. Given there were 6926 cases and 1695 of them had left herds which had a bTB breakdown within 450 days the PAF was calculated as 0.13 (95% CI: 0.12-0.14), or in terms of diseased cattle which leave donor herds and enter the risk window of recipients where they will disclose as bTB skin reactors or LRSs this has value (6926*0.13) 900 animals. If the 900 cattle had moved out to the same distribution of bTB breakdowns as all other case animals then 767 breakdowns could be attributed to this source, or 6.4% (767 of 12060 confirmed bTB breakdowns; 95% CI: 5.9-6.8) of confirmed bTB breakdowns during the nine year period of the study. Of the 12060 confirmed bTB breakdowns, 3695 had an associated risk window, meaning that 31% (95% CI: 29.8-31.5) of bTB breakdowns at disclosure contain an LRS(s) or bTB reactor(s) which had joined the herd within the previous 450 days. Given that 6.4% (95% CI: 5.9-6.8) of confirmed bTB breakdowns receive infected animal(s) in the 450 days prior to the herd disclosure, the calculated percentage range of total bTB reactors/ LRSs attributable to them was 1.1% (95% CI: 1.0-1.1) to 28.8% (95% CI: 28.5-29.1).

DISCUSSION

All confirmed bTB breakdowns in Northern Ireland currently receive an epidemiological investigation from their local VO as part of which a judgement is made as to the most likely infection source. Current figures indicate that approximately 14% of confirmed bTB breakdowns had purchased bTB infected animals as their infection source [18]. It must also be noted that the infection source could not be established in 31% of these confirmed bTB breakdowns [18]. Our result (6.4% (95% CI: 5.9-6.8)) suggested that this might be an overestimation for purchased infection as a bTB source. The fact that 31% of disclosing bTB breakdowns purchased bTB reactor(s) or LRS(s) within the previous 450 days may have biased this as choice of infection source.

Results of our study indicate that 6.4% (95% CI: 5.9-6.8) of confirmed breakdowns can be directly attributed to movement of infected animals. A similar value (determined using bTB herd restrictions) was found in ROI (Republic of Ireland) where 6-7% of current herd restrictions were attributed to the recent introduction of an infected animal [5]. Given the common environmental conditions and farm demographics between the ROI and Northern Ireland, agreement on this figure must provide some validation of the significance of this particular disease spread mechanism on the island of Ireland. The unique feature of our study design is the ability to control for the infection process which occurs after the animal leaves a donor/source herd. This design also allows quantification of infection levels in cattle as they move from donor to recipient herds.

When a herd purchases an infected animal, it has the potential to spread infection onwards to other cattle within that herd. Respiratory excretion and inhalation of *M. bovis* is considered to be the main route by which cattle to cattle transmission occurs in bovines [19]. Other routes of infection for bTB include ingestion of *Mycobacterium bovis,* which is considered a less common route, while udder infections which can lead to pseudo-vertical and congenital transmission are considered relatively rare [19, 20]. Excretion of the infectious agent *M. bovis* is an intermittent process with periods ranging from 6 to 25 weeks [19]. Also single time point sampling of nasal and tracheal mucus taken from reactors with grossly visible tuberculous lesions indicated that at least 19% were shedding *M bovis* [21]. Our study investigated movement of pre-infected animals; however of equal interest was the potential consequence of onward cattle to cattle transmission. The ability of an infected animal to disseminate infection is not a continuous process with some animals being more effective disseminators than others [19]. This suggests variability at a herd level in potential outcomes from receipt of infected animals. However, given the nature of our data, we can say that the range of proportion of bTB reactors/ LRSs attributable to purchased infected animals was between 1.1% (95% CI: 1.0-1.1) and 28.8% (95% CI: 28.5-29.1).

Movement of infected cattle is only one source of bTB for a herd. Within the UK wildlife mainly in the form of badgers plays a widely debated role in the transmission and maintenance of bTB. The direct contribution of badgers to confirmed bTB cattle herd breakdowns in high incidence areas in England has been estimated to be

5.7% (bootstrap 95% CI: 0.9-25%) with an overall contribution of badgers, taking into account onward cattle to cattle transmission, of 54% (overdispersion adjusted 95% CI: 38-66%) [22]. This work suggests that even though the level of transmission from badgers in these high incidence areas (5.7%), which is similar to the level of transmission to herds from purchased animals in our study (6.4%), the onward cattle to cattle transmission from a badger source appears to be greater. This partly could be a reflection of management practises where purchased animals such as beef fattening cattle are batched in such a way they have less access to other herd members reducing within-herd contact. Alternatively, farm biosecurity procedures may also limit within herd spread from purchased animals.

Finding an effective method to prevent infected animals being traded from one herd to another would assist in disease control. It would prevent bTB breakdowns directly instigated by movement of infected animals and prevent onward cattle to cattle transmission in these receiving herds. One such method to reduce the movement of bTB infected animals is a risk-based trading scheme where cattle herds are identified and ranked according to their risk of being or becoming infected [23, 24]. One such study, Adkin et al. identified herd history of bTB, local bTB incidence, herd size and movements of animals onto farms in batches from high risk areas as being significantly associated with increased probability of bTB infection on a farm [24]. The risk factors were then used to award each farm trading cattle in England and Wales a risk score (rating 1 – 5) with the aim of providing information allowing avoidance of risky purchases. These data are also readily available for Northern Ireland and a similar methodology could help reduce the number of bTB infected animals traded in Northern Ireland. Indeed, use of informed purchasing is a tool recommended within the recent bTB eradication strategy for Northern Ireland [25].

A limitation placed on this study is its dependence on information from the SICTT and abattoir surveillance. In Irish conditions, it was estimated that the sensitivity of the SICTT ranged between 68-95% while specificity ranged between 96-99%, although more recent analyses would suggest the sensitivity is lower than this [5, 26]. With abattoir surveillance, it has been reported that the standard post mortem examination has a low sensitivity (47%) for detection of tuberculous lesions [27]. The less than perfect sensitivity of both the SICTT and abattoir surveillance will lead to misclassification of infected animals as not infected, leading to an underestimate of the movement effect in this study. It is also important to note this study investigated cattle moving from source to receiving herds in officially recorded moves. Officially recorded movements were those notified by herd owners to DAERA, with the vast majority being sales of cattle directly to other farmers or through markets. Unofficial movements which could include practises such as unrecorded borrowing or lending of bulls are not factored into the design of this study. Unrecorded movements would lead to an underestimation of the movement effect in this study as they would initiate infections which would have then been attributed to other sources. Green et al. used a model to estimate movement based transmission of bovine tuberculosis in British cattle. In their work they stated that 9% of herd transmissions were unexplained, this group included infections where source was spread from unrecorded movements [6].

CONCLUSIONS

Farms in Northern Ireland tend to be geographically fragmented and frequently have a business model dependent on trading of live animals with obvious epidemiological disease consequences [3]. Veterinary investigations into the source of confirmed bTB breakdowns in Northern Ireland (2002 to 2015) indicated that no source was established in 31% of cases, 29% were due to local spread, 15% were due to badgers, 14% were due to purchased animals, 7% were due to recrudescence and 4% from other sources [18]. Although this study reported a lower percentage of breakdowns directly attributed to the purchase of bTB infected animals (6.4%), they still equated to almost one in fifteen bTB breakdowns, making this a significant source of infection. It is also important to acknowledge that purchase of infected animals will not always lead to the same outcome in different herds and at an individual herd level there could be great variability in the breakdowns initiated from this source. For this reason, it is important that policy makers take cognisance of this source of infection and apply rational options to combat it. Employment of mitigating measures such as risk-based trading options are an especially useful tool as they engender an empowered culture where decisions can be implemented based on fact.

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CONFLICT OF INTEREST

None.

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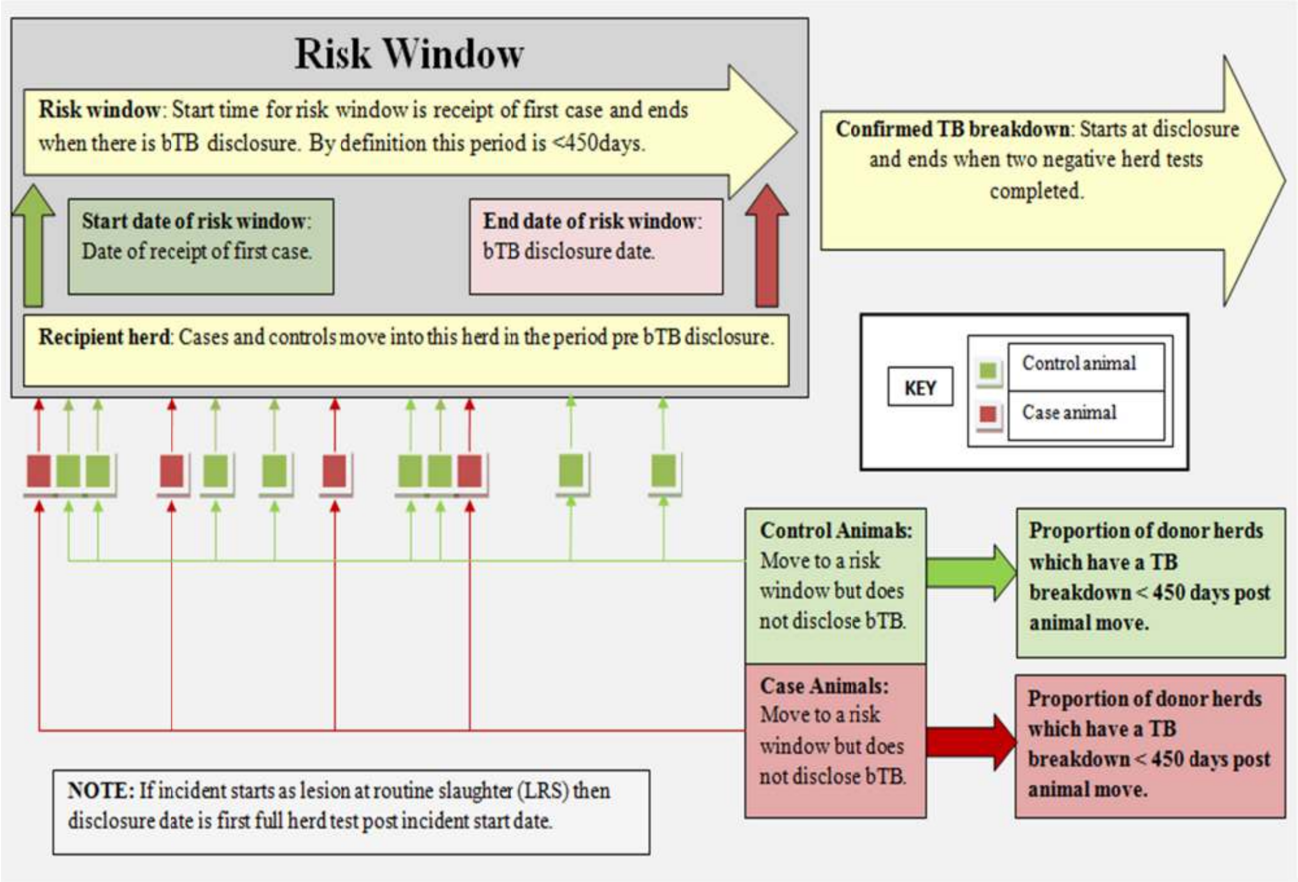
Figure1: Diagram showing study design for movement of cases and controls to recipient herd risk window.



Risk window: Start time for risk window is receipt of first case and ends when there is bTB disclosure. By definition this period is <450days.

Start date of risk window: Date of receipt of first case.

End date of risk window: bTB disclosure date.

Recipient herd: Cases and controls move into this herd in the period pre bTB disclosure.

Confirmed TB breakdown: Starts at disclosure and ends when two negative herd tests completed.

Control Animals: Move to a risk window but does not disclose bTB.

Proportion of donor herds which have a TB breakdown < 450 days post animal move.

Case Animals: Move to a risk window but does not disclose bTB.

Proportion of donor herds which have a TB breakdown < 450 days post animal move.

NOTE: If incident starts as lesion at routine slaughter (LRS) then disclosure date is first full herd test post incident start date.

Figure 2: Odds ratios for study variable bTB disclosure<450 days in the presence of an interaction with Number of days to closest bTB herd test after animal movement

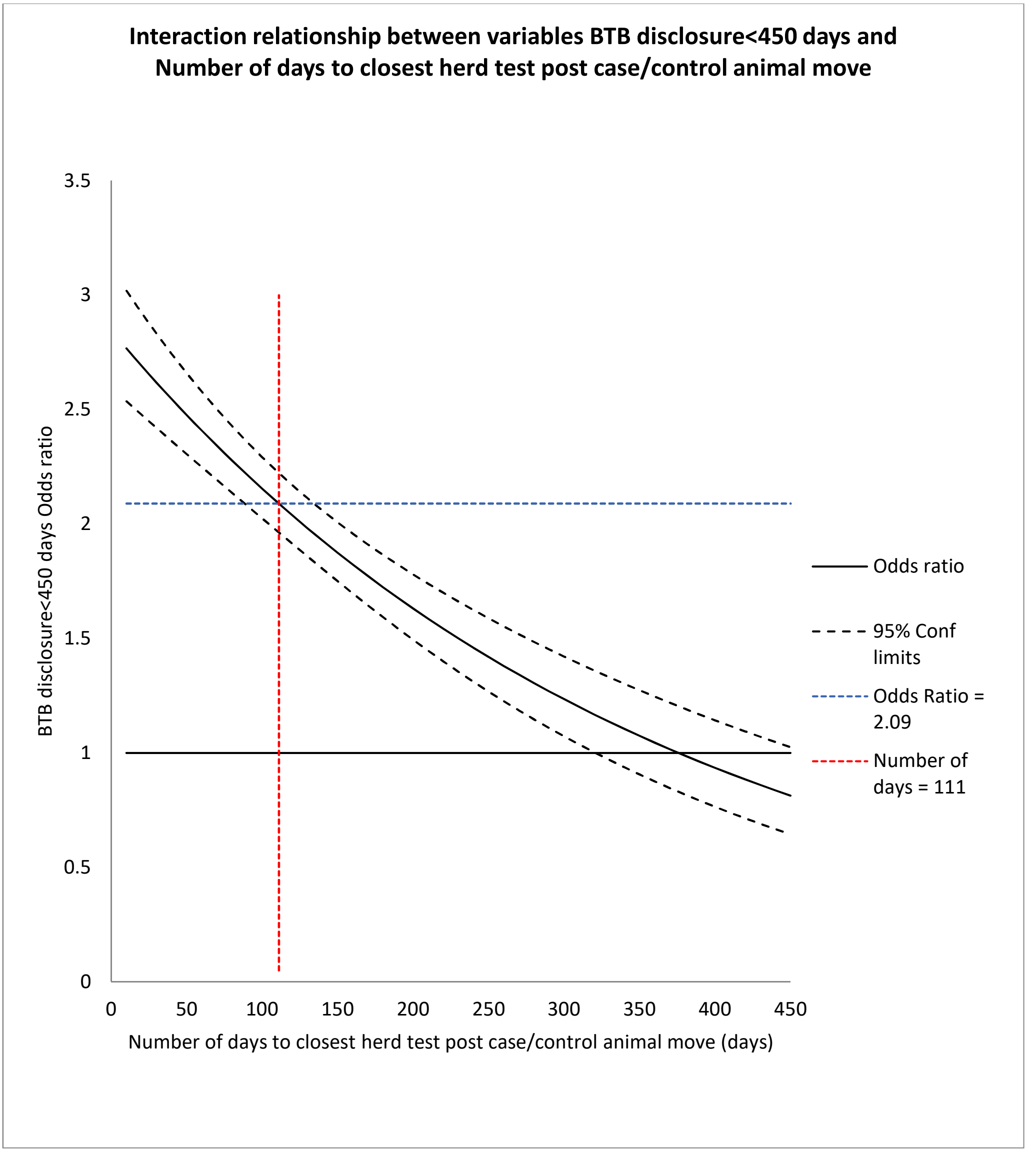
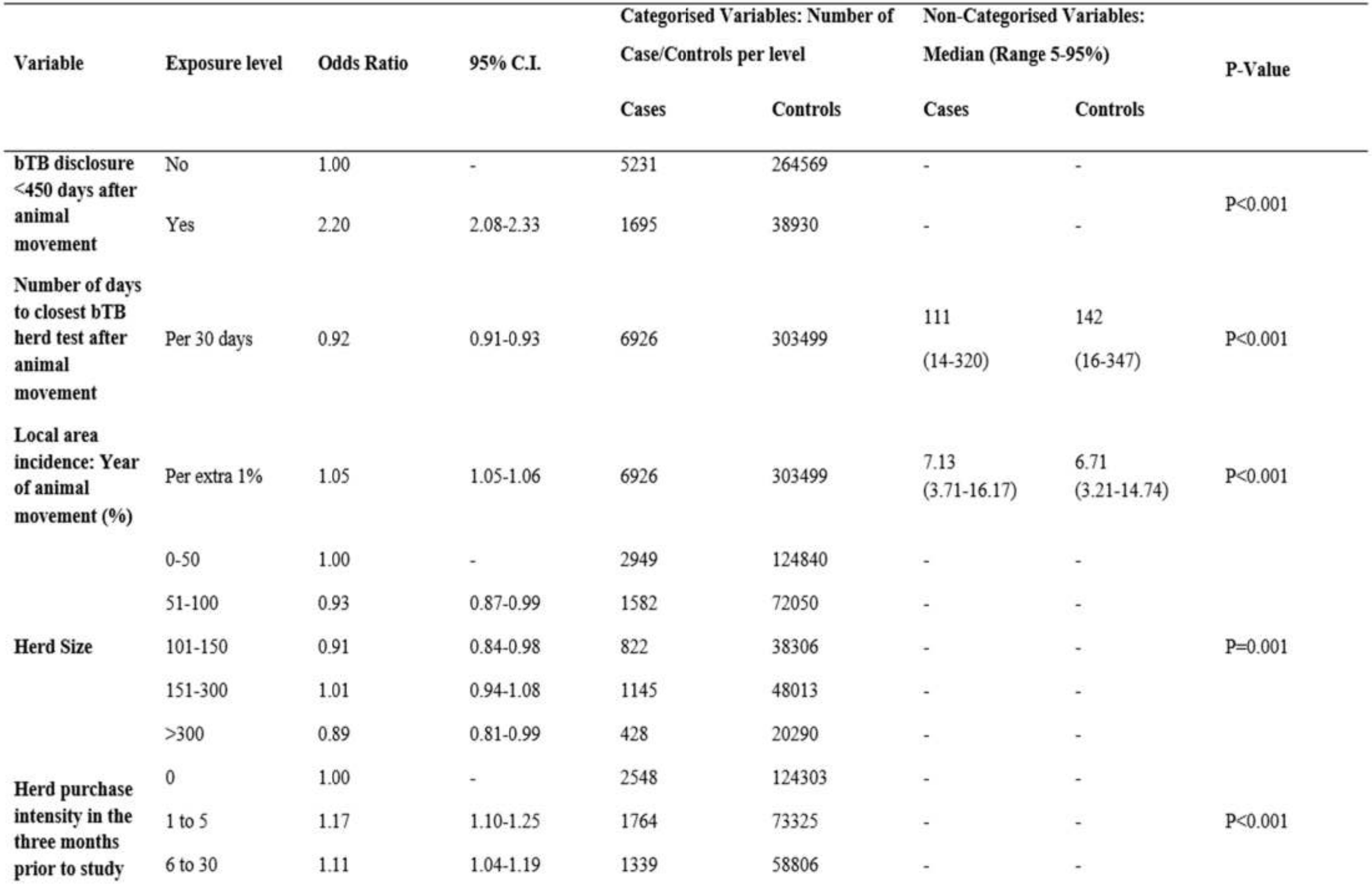


Table 1: Results of the univariable animal movement study with each of the explanatory variables used as a single fixed level term (DVO=Divisional Veterinary Office)



Variable	**Exposure level**	**Odds Ratio**	**95% C.I.**	**Categorised Variables: Number of Case/Controls per level**		**Non-Categorised Variables: Median (Range 5-95%)**		**P-Value**
				Cases	**Controls**	**Cases**	**Controls**	
bTB disclosure <450 days after animal movement	No	1.00	-	5231	264569	-	-	P<0.001
	Yes	2.20	2.08-2.33	1695	38930	-	-	
Number of days to closest bTB herd test after animal movement	Per 30 days	0.92	0.91-0.93	6926	303499	111 (14-320)	142 (16-347)	P<0.001
Local area incidence: Year of animal movement (%)	Per extra 1%	1.05	1.05-1.06	6926	303499	7.13 (3.71-16.17)	6.71 (3.21-14.74)	P<0.001
Herd Size	0-50	1.00	-	2949	124840	-	-	P=0.001
	51-100	0.93	0.87-0.99	1582	72050	-	-	
	101-150	0.91	0.84-0.98	822	38306	-	-	
	151-300	1.01	0.94-1.08	1145	48013	-	-	
	>300	0.89	0.81-0.99	428	20290	-	-	
Herd purchase intensity in the three months prior to study	0	1.00	-	2548	124303	-	-	P<0.001
	1 to 5	1.17	1.10-1.25	1764	73325	-	-	
	6 to 30	1.11	1.04-1.19	1339	58806	-	-	

Table 1 continued

Variable	Exposure level	Odds Ratio	95% C.I.	Categorised Variables: Number of Case/Controls per level		Non-Categorised Variables: Median (Range 5-95%)		P-Value
				Cases	Controls	Cases	Controls	
animal movement (animals/year)	31 to 100	1.10	1.01-1.21	557	24594	-	-	
	>100	1.56	1.44-1.69	718	22471	-	-	
Herd Type	Non-Dairy	1.00	-	5240	231052	-	-	P=0.368
	Dairy	1.03	0.97-1.09	1686	72447	-	-	
Presence of a bTB breakdown in the two years prior to study animal movement	No	1.00	-	5847	256056	-	-	P=0.910
	Yes	1.00	0.93-1.06	1097	47434	-	-	
DVO of herd of study animal	1	1.00	-	548	24519	-	-	P<0.001
	2	0.64	0.55-0.74	318	22342	-	-	
	3	0.93	0.82-1.05	704	34013	-	-	
	4	0.83	0.74-0.94	671	35977	-	-	
	5	1.35	1.21-1.51	1312	43395	-	-	
	6	0.82	0.72-0.93	471	25757	-	-	
	7	0.63	0.52-0.77	158	11133	-	-	
	8	1.40	1.25-1.56	1100	35152	-	-	
	9	1.49	1.32-1.68	716	21546	-	-	
	10	0.84	0.75-0.94	928	49665	-	-	

Table 2: Results from the final multivariable animal movement case control study (DVO=Divisional Veterinary Office)

Variable	Exposure level	Odds Ratio	95% Confidence Interval
bTB disclosure <450 days after animal movement	No	1.00	-
	Yes	2.85	2.60-3.12
Number of days to closest bTB herd test after animal movement	Per 30 days	0.95	0.94-0.96
[bTB disclosure<450 days] X [Number of days to closest bTB herd test after study animal movement]	Per 30 days	0.92	0.90-0.94
Local area incidence: Year of study animal movement (%)	Per extra 1%	1.02	1.01-1.03
Herd Size	0-50	1.00	-
	51-100	0.86	0.81-0.92
	101-150	0.79	0.73-0.85
	151-300	0.84	0.78-0.90
	>300	0.70	0.63-0.78
Herd purchase intensity in the three months prior to study animal movement (animals/year)	0	1.00	
	1 to 5	1.19	1.12-1.27
	6 to 30	1.13	1.05-1.21
	31 to 100	1.11	1.01-1.22
	>100	1.46	1.37-1.59
Presence of a bTB breakdown in the two years prior to study animal movement	No	1.00	-
	Yes	0.85	0.80-0.92
DVO of herd of study animal	1	1.00	-
	2	0.68	0.58-0.78
	3	0.95	0.84-1.08
	4	0.87	0.77-0.98
	5	1.35	1.21-1.51
	6	0.93	0.81-1.06
	7	0.72	0.59-0.87
	8	1.27	1.14-1.43
	9	1.35	1.18-1.53
	10	0.85	0.76-0.95

Chapter 5

Further description of bovine tuberculosis trends in the United Kingdom and the Republic of Ireland, 2003–2015

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The author of this thesis represented NI in this project and collaborated with the country representatives under direction from CVERA (Centre for Veterinary Epidemiology and Risk Analysis) at UCD (University College Dublin), ROI.

Further description of bovine tuberculosis trends in the UK and the Republic of Ireland, 2003-15

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Abstract

Extending on earlier work, trends in bovine tuberculosis (bTB) from 2003 to 2015 are described for the countries of the UK and the Republic of Ireland using standardised definitions and measures. Based on measures of animal and herd incidence, there remains a stable situation of extremely low prevalence in Scotland and the low risk area of England, and a higher but ongoing reduction in prevalence in Ireland. In Northern Ireland, there has been a rising bTB trend during 2010-15, although not to levels experienced during 2002-04. In the high risk and edge areas of England during 2010-15, the rising bTB trends have continued but with some recent evidence of improvement. In Wales, prevalence has fallen subsequent to a peak in 2008. The paper considers country-level differences in the light of key policy changes, which are presented in detail. This work is unique, and will assist policy-makers when critically evaluating policy options for effective control and eradication. Ongoing updates of this analysis would be useful, providing an evidence base for country-level comparison of bTB trends into the future. The use of multivariable analytical methods should be considered, but will rely on substantial sharing of raw data across the five countries.

Key words: Bovine tuberculosis, United Kingdom, Republic of Ireland, eradication, control

1. Introduction

Control of bovine tuberculosis (bTB, caused by infection with *Mycobacterium bovis*) is a major global challenge. Cattle are the primary host species, however, a number of other species, including the Eurasian badger (*Meles meles*), are important contributors to the epidemiology of *M. bovis* in cattle. bTB eradication has proved particularly challenging in the United Kingdom (UK, encompassing the countries of England, Northern Ireland, Scotland and Wales) and the Republic of Ireland (subsequently termed Ireland). Current control measures to prevent transmission from animals to the human population are generally effective, and zoonotic *M. bovis* infection in the UK and Ireland has occurred in a few isolated incidents and sporadic events.[1-4] During 2002-14, most cases in England, Wales, and Northern Ireland were in people greater than 65 years of age and born in the United Kingdom. Further, consumption of unpasteurised milk was identified as a risk factor among 74% of patients for *M. bovis* acquisition.[1]

In each of these countries, compulsory bTB eradication programmes commenced in the 1950s and are ongoing. Although each programme conforms to common EU legislation relating to surveillance and control measures, principally Directives 64/432/EEC[5] and 78/52/EEC,[6] country-level differences in programme strategy and management have emerged, reflecting political, geographical and other differences.[7] Each programme is underpinned by ongoing scientific research and there is an increasingly detailed understanding of opportunities and constraints to progress.

Abernethy and others investigated bTB trends in the UK and Ireland from 1995 to 2010.[7] In this earlier work, for the first time, standardised definitions and measures of bTB frequency and distribution were developed, which allowed progress of the disease eradication programmes to be evaluated across the five different countries. The work highlighted differences in cattle demography, bTB programme structure and test results, particularly between the island of Ireland (Ireland and Northern Ireland) and Great Britain (GB: England, Scotland and Wales). During this period, bTB trends indicated a stable situation of very low prevalence in Scotland and, over most of the period, a rising prevalence in England and Wales. The prevalence in Ireland declined, while Northern Ireland experienced both a rise and fall. The authors highlighted several issues requiring further consideration. Firstly, they proposed a review of the epidemiological basis of each of the bTB eradication programmes, and of underpinning legislation, to ensure that they are fit for purpose to adequately address risk factors for bTB. Secondly, they suggested that observed differences be formally explored to maximise the effectiveness and efficiency of each of the country programmes.

The current analysis is an extension of the earlier work by Abernethy and others.[7] Building on this earlier progress, we have extended both the period of interest (now to 2015) and measures of progress, now also incorporating the herd incidence density rate plus measures relevant to the management of bTB episodes, including frequency, duration and severity. As previously, we have sought to draw on the differing experiences and perspectives from each of the five countries. The objectives of the current study are to further describe bTB trends in the UK and Ireland, during 2003 to 2015.

2. Materials and methods

2.1 Glossary of terms

A glossary of terms is presented in Table 1.

Table 1. Glossary of terms

Abattoir case	A bovine animal, slaughtered under routine farm management, in which bTB was suspected following detection of suspicious lesions at slaughter and subsequently confirmed by laboratory testing (ie histological or bacteriological techniques). In GB, only bacteriological confirmation is used
Active herd	A herd engaged in cattle production (milk, beef, breeding)
Apparent animal prevalence	The sum of test reactors and abattoir cases as a percentage of the total cattle population
Apparent herd incidence	Percentage of active herds, unrestricted on 1st January, with at least one test reactor or abattoir case during a defined period (one year for annual herd incidence). See standardised herd incidence (SHI) for adjustments due to differences in mean herd size between countries
Apparent herd prevalence	Percentage of active herds with at least one test reactor or abattoir case during a defined period (one year for annual herd prevalence). See standardised herd prevalence (SHP) for adjustments due to differences in mean herd size between countries

Table 1 continued

bTB	“Bovine tuberculosis”, namely, infection of cattle with *Mycobacterium bovis*
bTB episode	Synonymous with bTB restriction
bTB-positive animal	Animal removed from a herd following a positive reaction to the skin test, or an abattoir case
bTB reactor	A bovine with a positive tuberculin skin (either standard or non-standard reactor) or interferon-ɣ blood test result that was removed from the herd. Animals with inconclusive results on two consecutive skin tests were included once this became policy (that is, from 2009 in Wales and 2010 in England and Scotland)
bTB restriction	A period during which out-movement of cattle is prohibited, other than to slaughter, due to presence of one or more reactors or abattoir cases. Synonymous with ‘bTB episode’
Confirmed abattoir case	An abattoir case from which tissue samples were positive for *M. bovis* on histology or bacteriology. In GB, only bacteriological confirmation is used
Confirmed reactor	A reactor to the skin test (or interferon-ɣ test in the UK) and in which bTB-like lesions were detected at post mortem examination or from which tissue samples were positive for *M. bovis* on histology or bacteriology. In GB, only bacteriological confirmation is used
Cumulative apparent animal prevalence	The animal-level prevalence used in the multiple year maps and calculated as the sum of reactors per hexagon divided by the sum of the mean population per herd per hexagon, each during a four-year period
Cumulative herd risk	The herd-level prevalence used in the multiple year maps and calculated as the percentage of herds in each hexagon with at least one bTB-positive animal in the four-year period
Herd incidence density rate	The total number of new bTB episodes that started during the year (the numerator) divided by ‘the time at risk’ (period when herd was not under bTB restriction, denominator)
Herd-size	Herd-size was calculated as the mean number of cattle tested at all whole-herd tests during the period in question.
Interferon-ɣ test	Diagnostic blood test that uses an enzyme-linked immunosorbent assay (ELISA) to detect interferon-ɣ, a component of cell-mediated immune response to *M. bovis.* The interferon-ɣ test is a supplementary ante-mortem test for bTB, generally used in confirmed bTB episodes, in conjunction with the skin test.
Inter-episode interval	The period between the start of the current restriction and the end of the previous restriction

Table 1 continued

Non-standard reactor	A bovine animal slaughtered as a reactor to the SICTT test, where the reaction to bovine tuberculin was 4mm or less than the reaction to avian tuberculin
OTF	Officially bTB free, as defined in Council Directive 64/432/EEC
OTFS	OTF status suspended, due to unresolved or as yet undetermined status pending a decision as to the true bTB status of the herd, or where all bTB-positive animals have no lesion or positive bacteriology. As defined in Council Directive 64/432/EEC
OTFW	OTF status withdrawn, due to presence of bTB in the herd. As defined in Council Directive 64/432/EEC
Prolonged restriction	By definition, a restriction of > 550 days in duration
Positive herd	A herd under bTB restriction
Restricted herd	A herd under bTB restriction
Restriction length	The number of days between the start and end of the current restriction
Short inter-episode interval	By definition, an inter-episode interval of ≤ 365 days
Six (6)-month post-restriction test	The whole-herd test conducted 6 months after OTF herd status has been reinstated
Skin test	The Single Intradermal Comparative Cervical Tuberculin Test (variously abbreviated as SICTT or SICCT), which is the primary ante-mortem test for bTB in the UK and the Republic of Ireland
Standard reactor	A bovine animal showing a positive response to the skin test where the reaction to bovine tuberculin was more than 4mm greater than the reaction to avian tuberculin
Standardised herd incidence (SHI)	As for apparent herd incidence, but adjusted for differences between countries in tested herd size by direct standardisation, using the sum of cattle populations across countries as the reference population
Standardised herd prevalence (SHP)	As for apparent herd prevalence, but adjusted for differences between countries in tested herd size by direct standardisation, using the sum of cattle populations across countries as the reference population
Study herd	Different definitions were used depending on the context, either *'Extending earlier measures of bTB frequency and recurrence'* or *'New descriptive statistics: frequency, duration and severity of bTB restrictions'*. See text for further details

2.2 Data

Data were obtained for the period 2003-2015. In Northern Ireland, bTB test data were extracted from the Animal and Public Health Information System (APHIS)[8]. In Ireland, bTB test data were obtained from the national database (Nixdorf for data prior to February 2005, the Animal Health Computer Information System

(AHCS) subsequently). Prior to analysis, these data were transformed into a bTB episode file, using methods as described previously[9] and as explained in detail below. In GB, bTB testing and post-mortem data were obtained from the Animal and Plant Health Agency's disease management system (Sam), and movement data were obtained from the British Cattle Movement Service (BCMS)'s Cattle Tracing System (CTS) to inform activity of herds.

2.2 Data analysis

2.2.1 General

Standardised case definitions were developed following detailed discussion within the project team, cognisant of country-level differences in data collection, management and interpretation. Some of these definitions had been agreed previously,[7] and new measures have since been defined. Country-level summary bTB statistics were then calculated separately in Belfast (for Northern Ireland), Dublin (for Ireland) and Weybridge (for GB: England, Scotland and Wales). For the period of interest (2003 to 2015), we calculated multiannual and annual summary statistics for all study herds, and by year, herd size and (in England) by risk area. The risk areas in England are geographically defined according to the nature of the bTB epidemic. In this paper, we present by the geographically defined areas throughout 2003-15, even though the risk areas did not come into active use, with area-specific strategic objectives and control policies, until January 2013.

These data were then collated for comparison and presentation. A range of software were used, including SAS v9.3 (in Ireland), SQL (in GB), and Microsoft Access^{TM} (Microsoft Corporation, Redmond, WA, USA, in Northern Ireland and GB) and StataIC 12 (in Northern Ireland).

2.2.2 Extending earlier measures of bTB frequency and recurrence

We used a number of summary statistics exactly as described previously by Abernethy and others,[7] including standardised herd prevalence, standardised herd incidence, apparent herd prevalence, percentage of new bTB episodes (unrestricted on 01 January that were subsequently restricted with bTB) that were detected by abattoir inspection, and percentage of herds positive at the 6-month post-restriction test.

In these calculations, study herds included all herds considered to be actively holding or trading cattle at the start of each year during the study period, as per the following definitions:

- That met at least two of the following criteria *(England, Scotland, Wales)*:
    - The herd was considered to be active according to administration dates at the start of the year,
    - The herd had a bTB test, a marked exempt test (a scheduled routine test that does not have to be performed because the herd is deemed low risk and therefore exempt from testing; in Scotland only), or an abattoir case during the study year depending on its area testing interval.
    - The farm (which may include a herd or herds over more than one location) had cattle in both the year of interest and the previous year according to the BCMS Cattle Tracing System.

- That presented cattle for bTB testing in the previous year or through which cattle moved in the previous two years *(Northern Ireland)*, or
- In which cattle were tested during the year or otherwise within the fifteen months prior to 1st January of that year *(Republic of Ireland)*.

In the previous study,[7] trends were described during 1995-2010. Here, these measures were updated to incorporate data from 2011 to 2015.

In addition, we calculated the herd incidence density rates during each year of interest as the total number of new bTB episodes that started during the year (the numerator) divided by 'the time at risk' (denominator). The time at risk was the sum of all time periods for all herds within the year of interest with the exception of periods of herd restriction (bTB episodes) as a consequence of bTB infection. Herds that were rendered inactive or going out of business during the year contributed to the time at risk for the full year. The herd incidence density rate was calculated as numerator/(denominator/365)*100, and expressed as restrictions per 100 herd-years at risk.

2.2.3 New descriptive statistics: frequency, duration and severity of bTB restrictions

A different definition for study herd was used for this aspect of the work. In all countries, a study herd included all herds with at least one test [any test: animal, part-herd, full-herd (in Scotland, also marked exempt test)] during each of the following periods: 2002-05 (2001-05 in England, Scotland and Wales on account of the foot and mouth disease epidemic), 2006-10, 2011-15, thus representing long established and consistently active herds.

A bTB episode is considered to encompass the period of herd restriction triggered by the discovery of infection, either as a bTB reactor or an abattoir case. Episodes are triggered either by field surveillance (commencing at the time of the disclosing test) or abattoir surveillance (commencing at the time that the abattoir lesion is disclosed), resulting in official bTB Free (OTF) status being either suspended (OTFS, unresolved or as yet undetermined status pending a decision as to the true bTB status of the herd) or withdrawn (OTFW, presence of bTB in the herd). An episode ends once OTF status is restored and the herd is eligible to trade. In the different countries, the period of herd restriction was calculated as follows:

- *In Northern Ireland*, a bTB episode is defined as a period of herd restriction during which infection is discovered either as a bTB skin reactor or an abattoir case. Episodes are initiated at the date a herd loses its OTF status and end at the point their OTF status is reinstated, which is concurrent with the herds eligibility to trade and, in the large majority of cases no more than a few days after the clearing test.
- *In Ireland*, a bTB episode begins when a herd loses its OTF status due to either a bTB skin reactor(S) or an abattoir case and ends following two clear consecutive bTB skin tests, with a minimum of a 60-day interval, the second of which must be carried out at a minimum of 4-months post removal of the last positive animal from the herd. Details of how the episode data were compiled are outlined by Gallagher and others[9] (in the supplementary material in that paper). For episodes triggered by abattoir surveillance the date of first full-herd test is recorded, whereas the date of lesion disclosure was not

available for all episodes. Therefore, a median time between the slaughter date of the positive animal and the first subsequent full-herd bTB test (so-called factory lesion test) was calculated each year for episodes where this information was available. For all episodes that were triggered by abattoir surveillance, the start of each episode was estimated as the date of the first full-herd test less this median time, appropriate to the year in question.

- *In GB*, bTB episodes start on the date a herd loses its OTF status following detection of a skin test reactor or abattoir case, and end when a TB10 legal notice is served to end restrictions and restore OTF herd status. The date of service of the TB10 notice can be recorded on the Animal and Plant Health Agency (APHA) Sam system any time from a few days to much longer after the clearing test, either due to administrative delays or the need for evidence that cleansing of the infected premises has taken place. To achieve alignment with Ireland and Northern Ireland, which mainly lift restrictions at the clearing test, the clearing test date has been used in GB where the restriction appears to have ended with this expected clear test, otherwise the official TB10 has been used. For the study herds from 2002, 95% of episodes end dates either were adjusted to the clearing test or were already officially set to the clearing test (88.5% adjusted).

Table 2 outlines the study population, the study period and the restriction of interest that were used when calculating measures of restriction frequency, duration and severity. In this paper, a prolonged restriction length was defined as > 550 days and a short inter-episode interval as ≤ 365 days.

Table 2. Measures of restriction frequency, duration and severity, including the relevant study population, study period and restriction(s) of interest. See glossary (Table 1) for definition of terms

	Study population				Restriction(s) of interest		
Measure	All study herds	Study herds with a restriction starting during the study period	Study herds with a restriction ending during the study period	Study period	All restrictions	All restrictions starting during the study period	All restrictions ending during the study period
Frequency							
Number of new restrictions							
Over the full 13 year period	✓			The full 13 year period		✓	
Each year	✓			Each year		✓	
Inter-episode interval (IEI)[i]							
IEI		✓		Each year		✓[ii]	
% IEI ≤ 365 days		✓		Each year		✓[ii]	
Duration							
Restriction length[iii]							
Restriction length			✓	Each year			✓
% restriction ≥ 550 days			✓	Each year			✓
Severity							
Total number of reactors and abattoir cases detected during the restriction[iv]			✓	Each year			✓

i. The IEI was not calculated for the first restriction during the study period. If the end of the last restriction is > 730 days prior to the start of the study period (1st Jan 2003), the days contributing to the inter-episode interval prior to 2003 will be left-censored at 730 days (i.e. 1st Jan 2001).

ii. The first restriction, if there is more than one during the study period

iii. In Northern Ireland, if the restriction started prior to 01 January 2001, the restriction length was left censored at 01 January 2001, in GB and Ireland no censoring was applied.

iv. Reactors and abattoir cases detected prior to 01 January 2003 were not included. In Northern Ireland prior to this date, it was difficult to accurately allocate them to an individual restricted period

2.3 Mapping

The techniques used to generate maps in this study were identical to methodology described previously.[7] These methods were established in an earlier collaborative exercise between the Republic of Ireland and Northern Ireland.[10] As described in Abernethy and others,[7] GB has a separate National Grid projection to that of the island of Ireland, thus, a map of the latter was transformed into World Geodetic System 84 (WGS84) format and then re-projected into British National Grid format. This caused a very slight distortion in the west/northwest of Ireland and, for this reason, a north arrow and graticule reference are not included in the maps. For aggregated spatial data, a uniform surface of 1542 hexagons, each with an effective diameter of 20 km, was created over the area of GB and the island of Ireland, using a custom script in ArcGIS 10.3 (ESRI, Redlands, California, USA). All farm points were then associated with the hexagon in which they were contained using a point in polygon analysis. Thereafter, animal and herd-level data for all farms were summarised for each hexagon, enabling the creation of thematic maps.

For the earlier measures of bTB frequency and recurrence, updated maps were created using the original methodologies, to enable a comparison between the previous two time periods presented in the manuscript by Abernethy and others[7]. The maps for the new descriptive statistics (frequency, duration and severity) are all drawn from the study population as described in Table 2, except that some denominators were adjusted to represent the positive population for the measure of interest, to emphasise differences in the infected population over time in isolation of the total population. This is noted in the description of the numerator and denominator in relevant Figures.

2.4 Policy changes

Substantial policy changes to the respective bTB eradication programmes during 2003-15 were documented by policy colleagues in each of the five countries and are summarised in Table S1 (online supplementary material).

3. Results

3.1 Demographics and geographical boundaries

The herd and cattle densities in 2012-15 are presented in the supplementary material (Figures S1 and S2), extending information for 1995-98 and 2006-09 presented previously (Figures 3 and 4, respectively, in Abernethy and others).[7] A map of the UK and the Republic of Ireland, including national boundaries and the bTB risk areas in England is presented in Figure 1.

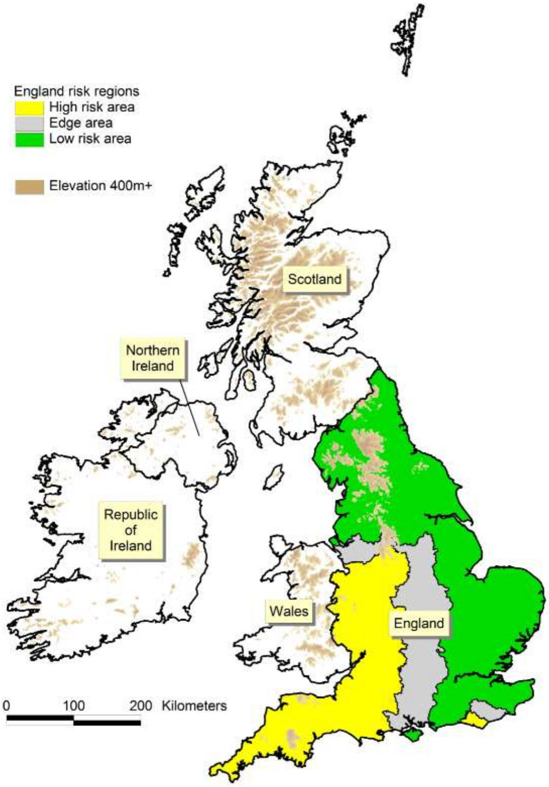

FIG 1: The UK and Republic of Ireland illustrating political boundaries and the bTB risk areas (low risk, edge, high risk) in England

3.2 bTB trends

3.2.1 Extending earlier measures of bTB frequency and recurrence

<u>i. Animal-level summary measures</u>

There are differing trends between countries in the proportion of the total cattle population with bTB disclosed (apparent animal prevalence) (Figure 2). In England, the apparent annual animal prevalence has increased steadily since 2001 in both the high risk area (HRA) and edge area, in 2015 to 0.93% and 0.16%, respectively. In Ireland, there has been a progressive decline, from a peak of 0.63% in 1999 to 0.26% in 2015. In Northern Ireland, there has been an increase in apparent annual animal prevalence in recent years, to 0.73% in 2015, from an earlier peak of 1.01% in 2003. In Wales, there has been a fall to 0.53% in 2015, from a peak of 0.83% in 2008. Apparent animal prevalence in the low risk area (LRA) of England and in Scotland has remained very low, reaching a maximum of 0.019% in 2015 and 0.015% in 2012, respectively. The cumulative apparent animal prevalence for 2012-15 (Figure 3) continues the earlier series for 1995-98 and 2006-09 (Figure 18 in Abernethy and others).[7] Comparing 2012-15 to 2006-09, in GB there has been ongoing expansion of the geographic area of highest cumulative apparent animal prevalence in the HRA of England. In Northern Ireland, areas of high cumulative apparent animal prevalence have persisted in the east and west of the country, whereas in Ireland there are now fewer areas of high cumulative apparent animal prevalence. The distribution of bTB reactors in 2014 is presented in Figure S3, extending earlier maps from 1997 and 2008 (Figure 19 from Abernethy and others).[7]

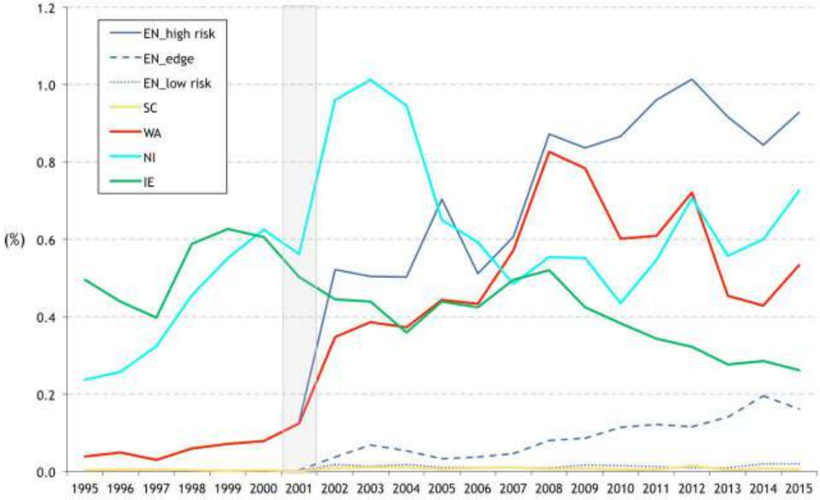

FIG 2: Annual apparent animal prevalence. The risk areas in England are only presented from 2001 *(a continuation of Figure 17 from Abernethy and others 2013).* The shaded area represents the duration of the foot and mouth epidemic

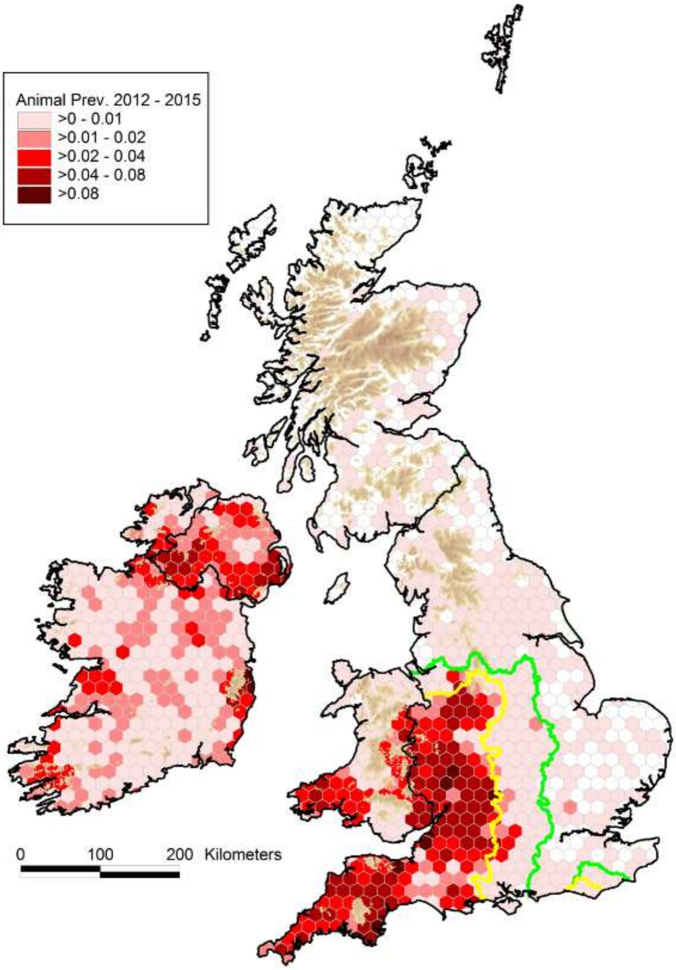

FIG 3: Cumulative apparent animal prevalence, 2012-15 *(sum of reactors (2012-15) per hexagon divided by the sum of the mean population per herd (2012-15) per hexagon) (a continuation of Figure 18 from Abernethy and others 2013).* The yellow and green lines delineate the boundaries between the high risk and edge areas, and between the edge and low risk areas, respectively

ii. Herd-level summary measures

The standardised annual herd prevalence (SHP) and standardised annual herd incidence (SHI) are presented in Figures 4 and 5, respectively, noting that the within-country trends in SHP and SHI are similar, apart from Northern Ireland where there has been an increase in SHI in recent years and a relatively stable SHP. In England, there has been a substantial increase in SHI during the 20 year period in both the HRA and edge area, albeit at a much lower level in the edge area. In the HRA and edge areas, SHI increased from 1.4% and 0.2% in 1995 to 12.5% and 4.0% in 2015, respectively. In England, there are signs in the HRA that the epidemic has stabilised to some degree. In Ireland, SHI has progressively fallen, from 6.5% in 1995 to 3.7% in 2015.

Northern Ireland experienced a rise in SHI from 5.4% in 1995 to 11.7% and 11.4% in 2002 and 2004, respectively, followed by a fall to 6.8% in 2010. Subsequently, the SHI has again risen, to 10.3% in 2015, but not to levels experienced during 2002-04. Throughout the period, SHI has not exceeded 0.4% in Scotland and 0.7% in the LRA of England. In Wales, SHI rose to 8.2% in 2009 from 0.8% in 1995, but has since fallen, to 6.3% in 2015 (Figure 5). The annual herd incidence density rate in the HRA of England almost doubled between 2003 (8.7 cases per 100 herd-years at risk) and 2015 (16.8), and in 2015 was almost twice that experienced in that year in Northern Ireland (8.3) and Wales (7.6). There has been ongoing geographic expansion of areas of high cumulative herd risk from 1995-98 (Figure 14 in Abernethy and others)[7] through to 2012-15 (Figure 7).

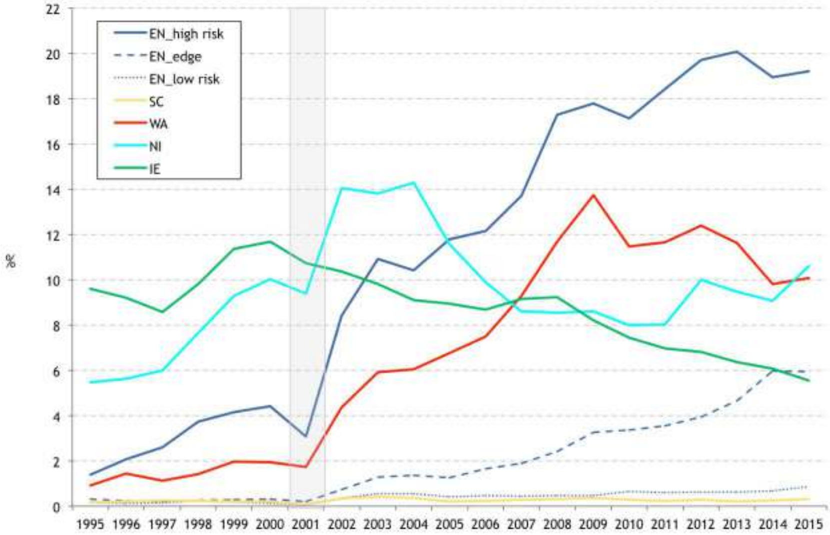

FIG 4: Standardised annual herd prevalence *(a continuation of Figure 9 from Abernethy and others 2013).* The shaded area represents the duration of the foot and mouth epidemic

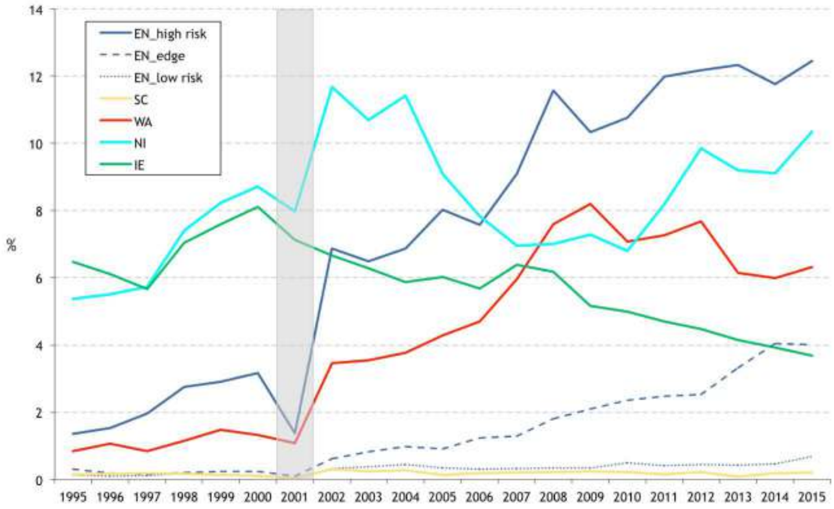

FIG 5: Standardised annual herd incidence *(a continuation of Figure 11 from Abernethy and others 2013)*. The shaded area represents the duration of the foot and mouth epidemic

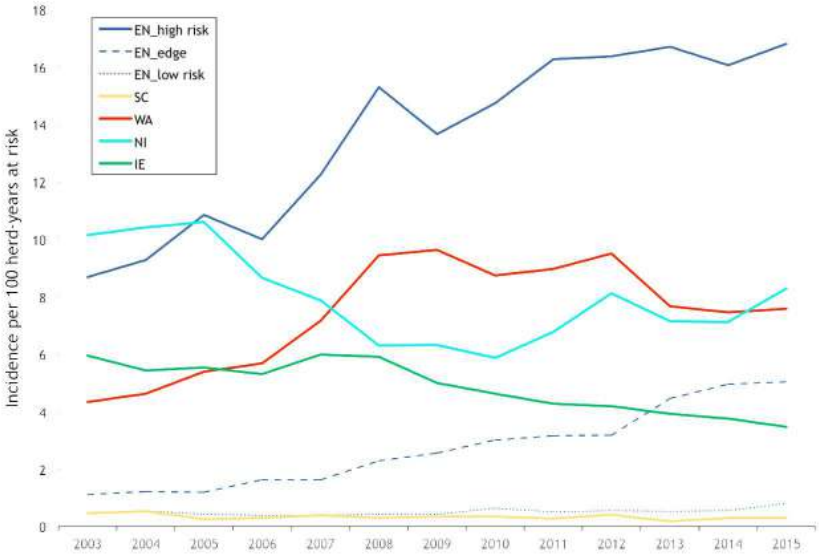

FIG 6: Annual herd incidence density rate per 100 herd-years at risk

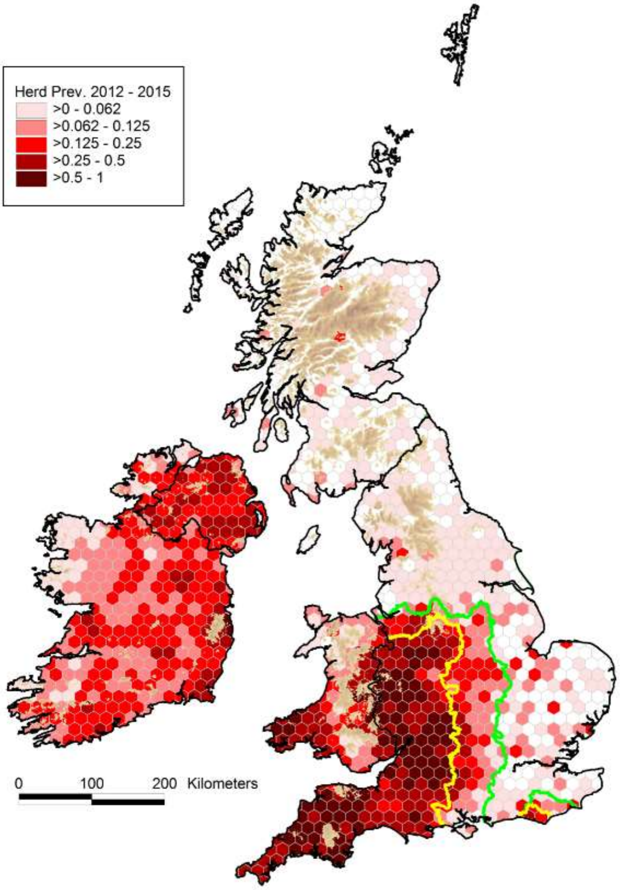

FIG 7: Cumulative herd risk, 2012-15 *(count of herds with ≥ 1 reactor for the period 2012-15 divided by count of active herds per hexagon 2012-15) (a continuation of Figure 14 from Abernethy and others 2013).* The yellow and green lines delineate the boundaries between the high risk and edge areas, and between the edge and low risk areas, respectively

iii. Additional measures

The percentage of bTB restrictions detected by abattoir surveillance is presented in Figure 8. Although Ireland and Northern Ireland were similar up until 2001, Ireland is now substantially different to each of the other countries. During 2005 to 2015, between 26.8% (in 2012) and 36.4% (2006) of bTB restrictions in Ireland were detected by abattoir surveillance. In each of the other countries and bTB risk areas, the equivalent figure is much lower: in 2015, the equivalent percentage was 15.8% (Northern Ireland), 15.4% (England, LRA), 14.9% (England, HRA), 11.5% (England, edge area), 10.3% (Scotland) and 8.3% (Wales).

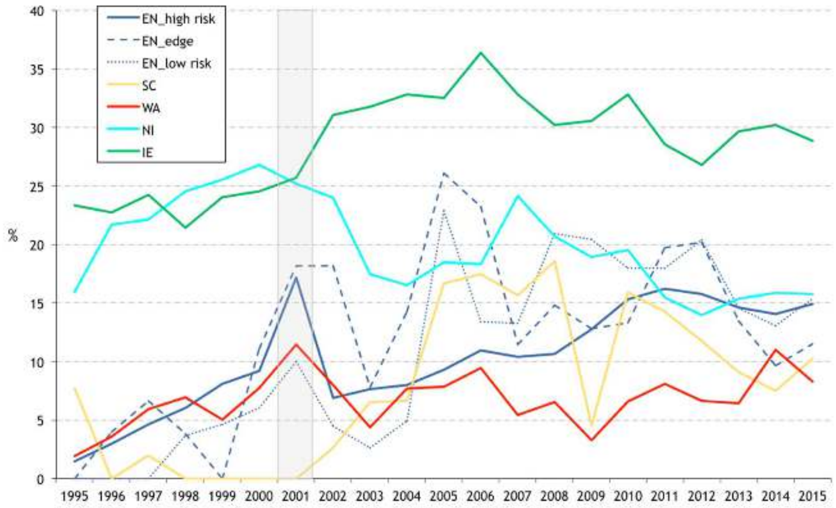

FIG 8: Percentage of bTB restrictions detected by abattoir surveillance *(a continuation of Figure 23 from Abernethy and others 2013).* The shaded area represents the duration of the foot and mouth epidemic

The percentage of herds positive at the 6-month post-restriction test is highest in the HRA of England, being 17.8% in 2015 (Figure 9). In most other countries and bTB risk areas, the breakdown rate is much lower: 10.0% in the edge area of England, 9.6% in Wales during 2015 and 9.4% in Ireland during 2015. In Northern Ireland, however, the situation has worsened between 2013 and 2015, with 13.4% of herds positive at the 6-month post-restriction test in 2015. In both Figures 8 and 9, low herd numbers in Scotland and parts of England (the LRA, also the edge area to some degree) have resulted in a more erratic trendline.

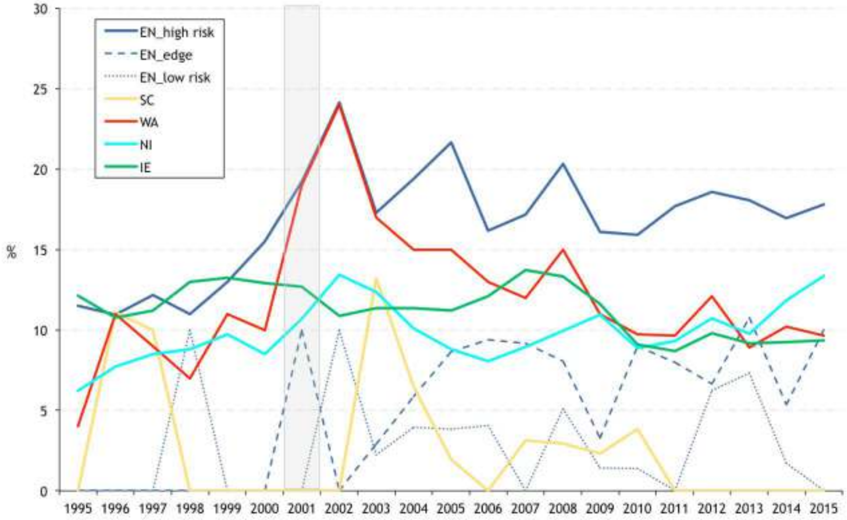

FIG 9: Percentage of herds positive at the 6-month post-restriction test. No data are presented for Scotland after 2010, where the 6-month post-restriction test was abolished for OTFS (officially bTB free status suspended) unconfirmed episodes *(a continuation of Figure 25 from Abernethy and others 2013).* The shaded area represents the duration of the foot and mouth epidemic

3.2.2 New descriptive statistics: frequency, duration and severity of bTB restrictions

i. Study population comparison

There were fewer herds that met the study herd definition for the new descriptive statistics as described in section 2.2.2 (frequency, duration and severity of restrictions) compared to the number of study herds used for the previous measures as described in section 2.2.1 (extending existing measures), as illustrated in Figure S4 (online supplementary material). To quantify the extent and the spatial distribution of herds excluded due the herd selection criteria in the new measures versus the previous measures study populations, a number of spatial calculations were performed (Figure S4 (a to f)). Figure S4 (a to c) show the count of herds as per selection criteria for the previous measures as a single year, 2014, (a) and as a rolling period, 2012-2015, (b) and for the new measures period, 2003-2015, (c). The selection criteria yielding the highest herd count per hexagon (a) represents herds that were registered as active in 2014 (213,802 herds). Figure S4(b) shows the number of herds per hexagon that returned a bTB test result in the period 2012-2015 (197,552 herds) selected from herds defined as active in 2014, Figure S4(a). Figure S4(c) shows the number of herds per hexagon as per the new descriptive measures selection criteria for 2003-2015 (184,414 herds). The differences in herd counts per hexagon according to selection criteria are displayed in Figure S4(d) and (e); Figure S4(d) showing the number of herds less per hexagon between the selection criteria with the highest count (a) and the lowest count (c); and Figure S4(e) showing the difference between herds active in 2014 requiring a bTB test between 2012-2015 (b) and the 2003-2015 (c) period. Finally, Figure S4(f) shows the approximate number of cattle excluded from the new descriptive measures population when compared with all active herds in 2014 (3,464,980). In the new descriptive statistics, there were fewer study herds per hexagon in large parts of Scotland and the LRA of England. The difference in herds per hexagon when comparing all active herds on January 1st 2014 to those when applying the new measures criteria (2003-2015) are presented as a percentage herd loss in Figure S5 (online supplementary material).

ii. Frequency

Number of restrictions

The mean number of restrictions per study herd during 2003-15, by herd size, is presented in Figure 10, and the mean number of restrictions per study herd annually in Figure 11. There is an increase in the mean number of restrictions with herd size (Figure 10), even in areas of low bTB risk, indicating that herd size is a risk factor for bTB restrictions in all areas and countries. In the HRA of England, there has been an increase in the mean number of restrictions per herd annually from 0.101 in 2003 to 0.170 in 2011 then a steady fall to 0.157 in 2015, whereas the edge area has continued to rise from 0.016 in 2003 to 0.055 in 2015 (Figure 11). In 2015, the mean number of restrictions per herd was substantially higher in the HRA of England (0.157) compared to Northern Ireland (0.083) where it has been rising, and Wales (0.075) and Ireland (0.034), which have both been decreasing. Figure 12 presents the mean number of restrictions per study herd per hexagon during 2003-15, for herds with at least one restriction during this period. Among these herds during this period, the mean number of restrictions was greatest in the southwest of Wales and most of the HRA of England, indicating a high level of recurrence, more so than that observed in Ireland and Northern Ireland.

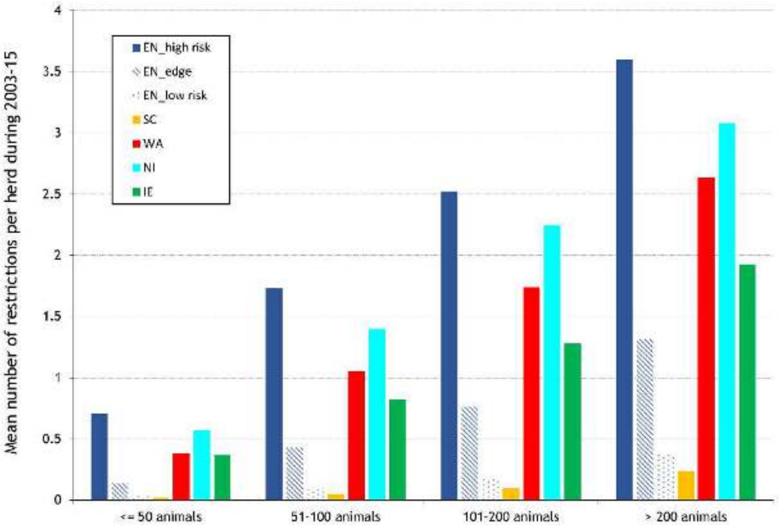

FIG 10: Mean number of restrictions per study herd during 2003-15, by herd size

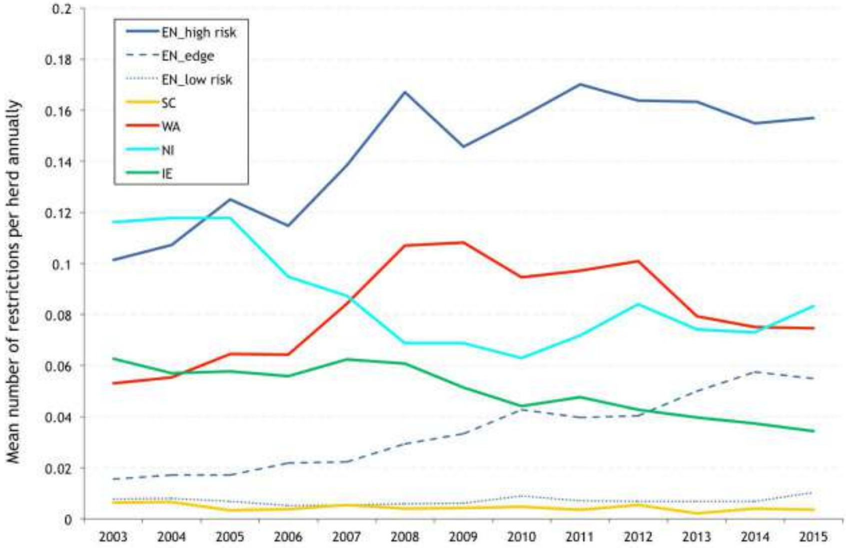

FIG 11: Mean number of restrictions per study herd annually

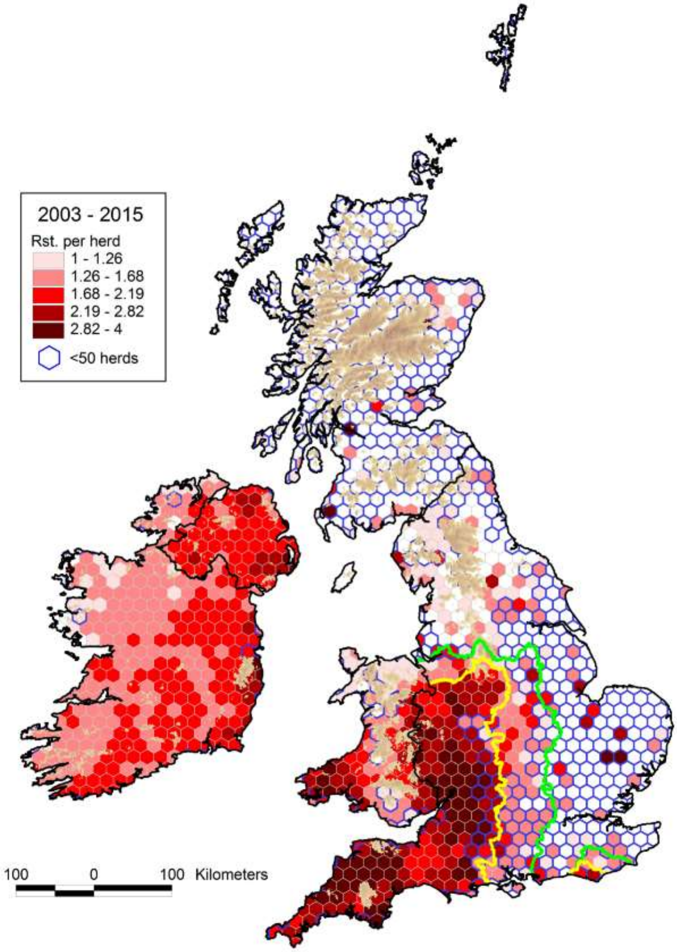

FIG 12: Mean number of restrictions per study herd per hexagon during 2003-15, for those study herds with at least one restriction during this period *(total restrictions per hexagon for 2003-15 divided by the number of herds with a restriction per hexagon 2003-15).* The yellow and green lines delineate the boundaries between the high risk and edge areas, and between the edge and low risk areas, respectively

Inter-episode interval

The annual percentage of study herds restricted during the year of interest with a short inter-episode interval (that is, ≤ 365 days) is presented in Figure 13. During 2003-15, there has been a general fall in the percentage of herds restricted with a short inter-episode interval across all countries and areas although the trend line is erratic in low bTB areas. In 2015, this was 33.3% in edge area of England, 30.5% in the HRA of England, 19.0% in Northern Ireland, 18.5% in Wales, and 13.5% in Ireland (Figure 13). Figure 14 presents the mean inter-episode interval per hexagon during 2005, 2010 and 2015, but limited to those study herds that were restricted during the year of interest with an inter-episode interval ≤ 730 days.

FIG 13: Annual percentage of study herds restricted during the year of interest with a short inter-episode interval (≤ 365 days)

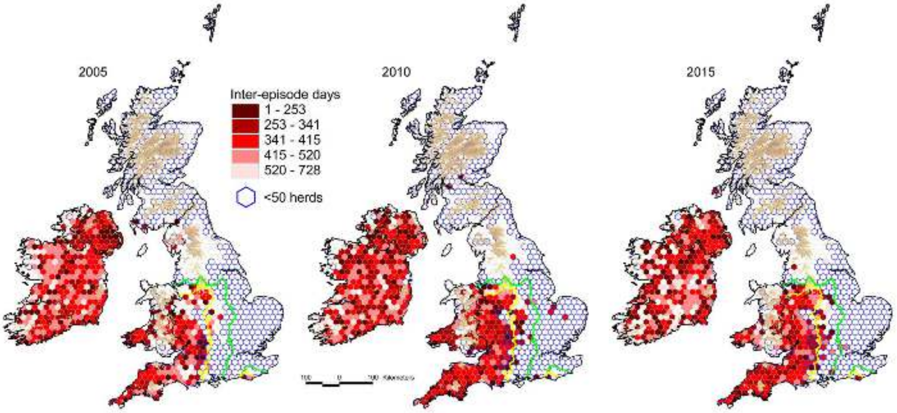

FIG 14: Mean inter-episode interval per hexagon during 2005, 2010 and 2015, for those study herds that were restricted during the year of interest with an inter-episode interval ≤ 730 days. The yellow and green lines delineate the boundaries between the high risk and edge areas, and between the edge and low risk areas, respectively

iii. Duration

The median restriction length for restrictions ending during the year of interest is presented in Figure 15, and the percentage of prolonged restrictions (> 550 days) in Figure 16. In 2015, the percentage of prolonged restrictions was highest in the HRA of England (6.4%), Wales (5.1%) and Northern Ireland (4.3%), and consistently less over the study period in the edge area and LRA of England (2.3% and 0.93% in 2015, respectively) and in Ireland (1.4%). During 2011-15, there were no prolonged restrictions in Scotland (Figure 16). Figure 17 presents the mean restriction length, by hexagon (top) and percentage of prolonged restrictions (> 550 days), by hexagon (bottom), during 2007, 2011 and 2015.

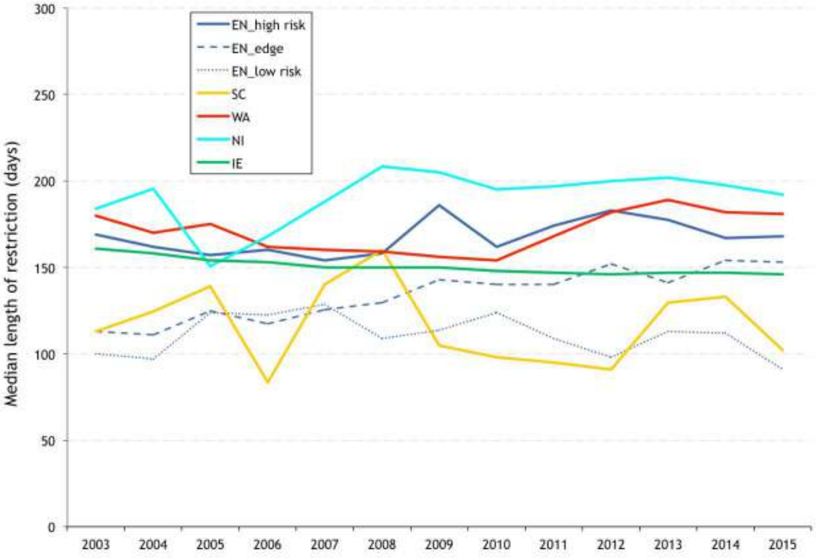

FIG 15: Median restriction length for restrictions ending during the year of interest

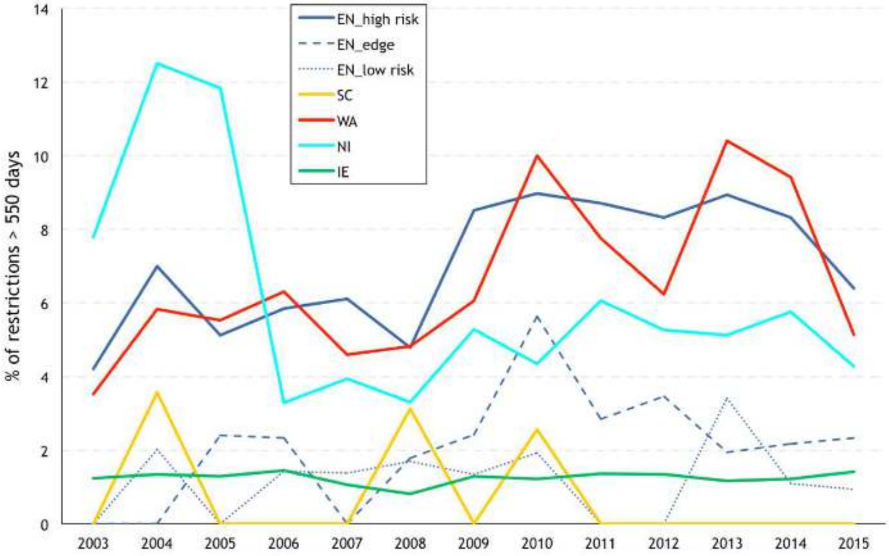

FIG 16: Percentage of restrictions > 550 days in duration (prolonged restrictions)

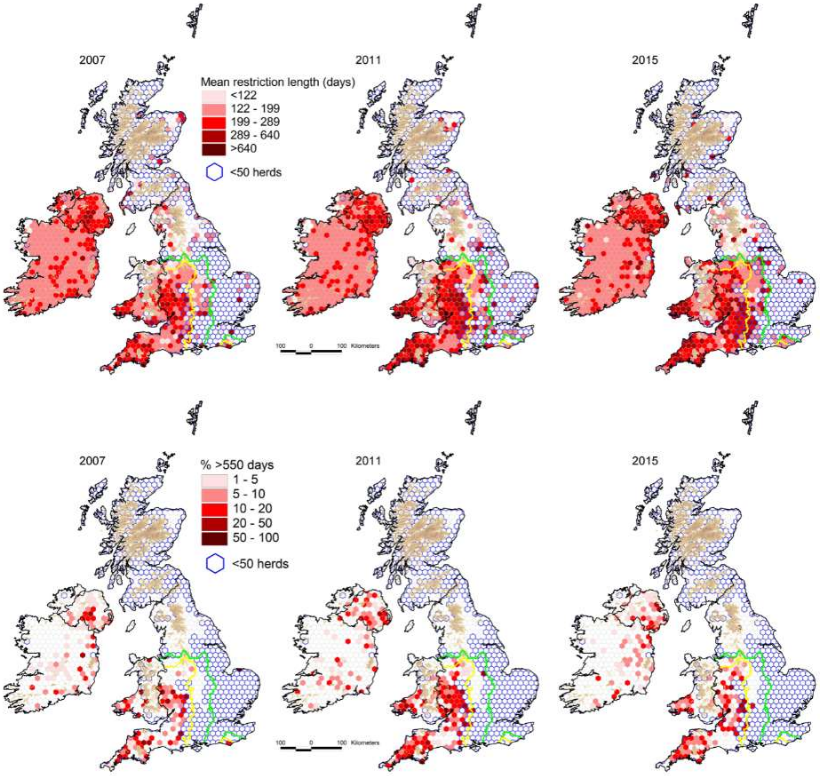

FIG 17: Mean restriction length, by hexagon (top) and percentage of prolonged restrictions (> 550 days), by hexagon (bottom), during 2007, 2011 and 2015 *(restriction length per hexagon divided by the number of restricted herd per hexagon 2007, 2011 and 2015 (top), percentage of restricted herds with a restriction length > 550 days per hexagon 2007, 2011 and 2015 (bottom)).* The yellow and green lines delineates the boundaries between the high risk and edge areas, and between the edge and low risk areas, respectively

iv. Severity

The mean number of reactors and abattoir cases per restriction, limited to study herds with a restriction ending during the year of interest is presented in Figure 18. In comparison to 2005, this number was higher in 2015 in high risk (6.1 in 2005 and 7.7 in 2015) and edge (3.8 and 9.8) areas of England, but not in other countries and bTB risk areas: the LRA of England (3.5 and 3.7), Ireland (3.9 and 4.0), Northern Ireland (5.3 and 5.9), Scotland (5.3 and 4.1) or Wales (6.1 and 6.7), although a rising trend in Wales had been observed up to 2013. The mean number per restriction per hexagon, in 2007, 2011 and 2015, is presented in Figure 19.

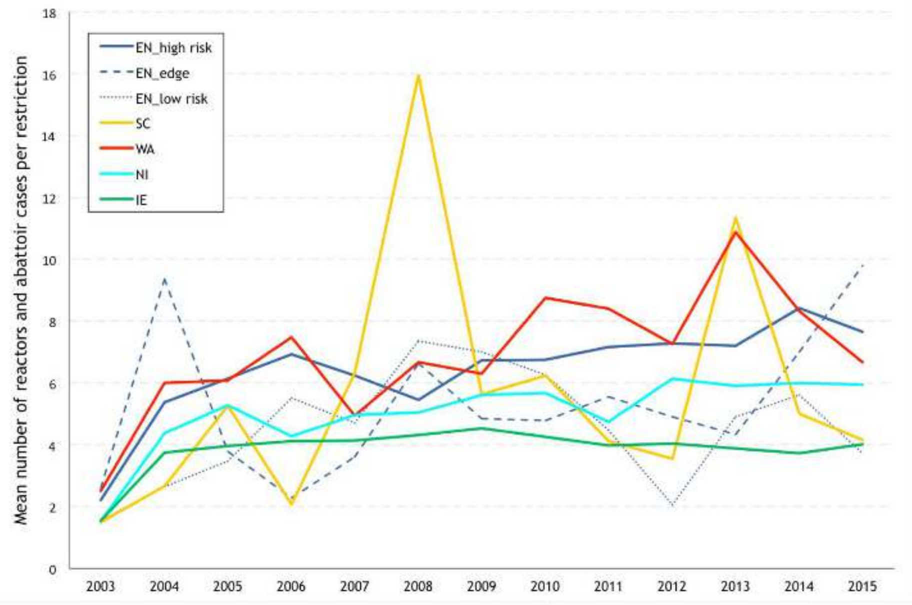

FIG 18: Mean number of reactors and abattoir cases per restriction, limited to study herds with a restriction ending during the year of interest

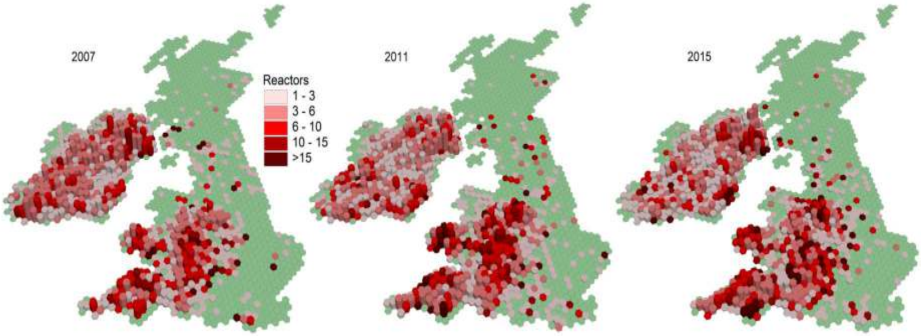

FIG 19: Mean number of reactors and abattoir cases per restriction per hexagon, in 2007, 2011 and 2015. For each hexagon, colour is used to indicate the mean number of reactors and abattoir cases per restriction, and column height to reflect the total number of study herds with a restriction ending during the year of interest

3.3 Policy changes

A summary of current policy and a timeline of substantial policy changes relating to bTB surveillance and control in the five countries during 2003-15 are presented in Table S1 (online supplementary material).

In 2013, England was regionalised for bTB surveillance purposes into a Low Risk Area (LRA) of background four-yearly herd testing counties and a High Risk Area (HRA) of annually tested herds, separated by a continuous 'buffer' zone known as the Edge Area where herds are tested every six or twelve months (Figure 1). Building on this, a new strategy was launched in April 2014 with the aim of gradually achieving officially bTB free (OTF) status for the whole country by 2038. The strategy takes account of the heterogeneous spatial distribution and epidemiology of the disease across the country. It consists of a comprehensive package of measures to tackle all sources of infection for cattle, including progressively more frequent and sensitive herd testing regimes, tighter cattle movement controls (e.g. pre- and post-movement testing), enhancing farm biosecurity and resilience to infection risks, support for injectable vaccination of badgers in the Edge Area and licensed culling of badgers in the HRA and certain parts of the Edge Area, where this species acts as a true maintenance host of *M. bovis* infection.

Ireland has introduced a comprehensive wildlife control programme based on population reduction in areas where epidemiological investigation has identified wildlife as likely source of infection in cattle herd breakdowns.[11] Science-based advice has steered advances in policy decisions,[12] and an evolving quality control system on all elements of the programme has been introduced.

In contrast to Ireland, Northern Ireland has not implemented a wildlife control programme but instead focussed efforts on cattle controls. The measures introduced over the duration of this study which are considered to be the most important in reducing the spread of TB were the automatic ban on moves out of herds with overdue TB tests (2004), the introduction of interferon-ɣ testing as an integral part of the bTB programme (2007), and the removal as reactors those animals that give an inconclusive result to a second consecutive bTB test (2010)

Scotland has maintained a relatively low and stable incidence of bovine tuberculosis over the period, which probably represents the lowest baseline incidence that is consistent with maintaining risk-mitigated trade with the rest of the UK, where incidence is significantly higher. There remains no evidence of significant wildlife reservoirs of infection to cattle in Scotland, as evidenced by the absence of recurrence of disease on farms and the absence of breakdowns on neighbouring farms. As such Scotland's policies concentrate on early identification and eradication (pre and post movement testing of cattle coming to Scotland from high risk areas since 2005 and mandatory supplementary gamma interferon testing in any new confirmed (OTFW) breakdowns since 2006). Since the introduction of gamma interferon there have been no herd slaughters for bovine tuberculosis in Scotland. The final policy with a significant impact has been the introduction of risk-based routine surveillance, to minimise unnecessary testing in the lowest risk herds, although the impact is related to surveillance efficiency rather than reduced incidence.

After the introduction of annual testing in 2010, Wales TB control policy sought to address TB test sensitivity, expansion of cattle controls and enhanced management of prolonged breakdowns. Policy changes which have come into effect in the depicted supervisory period include the withdrawal (rather than suspension) of Officially TB Free (OTF) status on epidemiological grounds other than confirmation of disease; necessitating 2 clear tests after most TB breakdowns (Oct 2011). Since 2012, the Intensive Action Area, the area with the highest TB breakdown density in Wales, has seen 'combined cattle controls' incorporating the deployment of a TB badger vaccination programme (until 2015), passive badger surveillance and 6 monthly TB testing. In 2014, the All Wales Badger Found Dead Survey commenced, the largest passive surveillance programme of bTB in wildlife in Wales to date, which has fed into bTB policy decision-making. In the same year, the enhanced management of persistent breakdowns (EMPB) was initiated, entailing among others, enforcement of biosecurity measures and an immediate increase in the use of targeted gamma interferon testing.

4. Discussion

Among policy makers, there is considerable interest in comparison of bTB trends across countries. Indeed, this information is needed to facilitate critical evaluation of policy options for effective control and eradication. This study, and the earlier work by Abernethy and others,[7] are unique, offering an example of harmonised approaches to measuring progress towards bTB control and eradication across a number of countries. The approach is consistent with recommendations from the European Commission[13] which highlighted the importance of epidemiological data analysis using agreed epidemiological indicators for continuous assessment and subsequent enhancement of an eradication programme. Extending on the earlier work,[7] we describe bTB trends in the UK and Ireland during 2003 to 2015. We extend both the period of interest (previously to 2010, now to 2015) and the measures of progress, which now include the herd incidence density rate plus measures relevant to the management of bTB episodes, including frequency, duration and severity. England is now presented as three risk areas (HRA, edge area, LRA), which were outlined in 2011[14] and in active use, with area-specific strategic objectives and control policies, from January 2013. Further, in Table S1 (online supplementary material) we document current policy and a timeline of substantial policy changes relating to bTB surveillance and control in the five countries during 2003-15. We also draw on the differing experiences and perspectives across the five countries.

We have introduced a number of new measures of progress, relating to restriction frequency, duration and severity. Whereas incidence and prevalence report absolute numbers at an area level, these new measures place greater focus in each area on the burden of disease at the farm level, such as how many animals they are losing?, how long farms are under restrictions?, and how many times farms are under restriction? Nonetheless, the new measures relevant to the management of bTB episodes need to be interpreted with care, for several reasons. The two measures of frequency (number of new restrictions, inter-episode interval) are associated, as are these measures of frequency and duration (restriction length). Increasing restriction frequency would generally be associated with decreasing inter-episode interval, and restriction frequency should decrease with increasing restriction length. Similarly, with a general (i.e. national) increase in bTB, restriction frequency will increase and inter-episode interval will decrease, given that bTB clusters in the same herds.[15] In addition, some of the measures are influenced by factors related both to disease management and disease control. If there were a national decision to extend restriction length, for example under risk-averse management, this would likely lead to a decrease in the number of new restrictions per unit time and the potential for increase in inter-episode duration, as has occurred to some extent in the more chronic areas with increases to the number of control tests and a more severe interpretation of skin testing. This effect is ameliorated to a large degree, but not completely, given the application under common EU legislation[5 6] of similar cattle controls in each country. We contend that these complexities are a feature of bTB programmes, where multiple measures are useful when critically evaluating programme progress. There is a need to collectively consider trends across a range of measures and to account for the direct impact on these measures of any management decisions. Therefore, the measures need to be interpreted collectively and with care.

In this study, the temporal trends were undertaken using country-level aggregate data. The work was undertaken separately at three centres (Belfast, Dublin, London), working with different IT systems and national databases, to develop agreed summary measures of trends in time. As mentioned previously, standardised case definitions were developed following detailed discussion within the project team, cognisant of country-level differences in data collection, management and interpretation. A different approach was used for the spatial trends, noting the development of maps to a much finer scale. Here, under appropriate data sharing agreements, herd-level data were shared, which allowed us to create polygon-level spatial summary statistics. Using this approach, there were limited opportunities for detailed data analysis. Standardised herd incidence and herd prevalence were calculated to adjust for differing herd demographics (specifically herd-size) and time at risk, across the five countries.

In the preceding paper, covering bTB trends in the five countries during the period from 1995 to 2010, Abernethy and others[7] highlighted a stable situation of extremely low prevalence in Scotland, a higher but reducing prevalence in Ireland, and, up to 2009, rising trends in England, Wales and Northern Ireland. In this current work, this situation has continued during 2010-15 in Scotland, the LRA of England and Ireland, including ongoing falls in both animal (Figure 2) and herd (Figure 4) prevalence in Ireland. In the HRA and edge area of England during 2010-15, the rising bTB trends have continued, as highlighted in measures of animal and herd prevalence and herd incidence (Figures 5 and 6), albeit at a much lower level in the edge area. However, there are signs of stabilisation of trends in these measures in the HRA. In Northern Ireland, there has been a rising bTB trend during 2010-15, although not yet back to levels experienced during 2002-04. In Wales, animal and herd prevalence have each fallen subsequent to a peak in 2008.

The new measures of restriction frequency, duration and severity generally follow the same patterns as animal and herd prevalence and incidence. Each reflects an improving situation, though due to the cyclical nature of many of these trend lines, a sustained fall over several years may need to be observed to have confidence in these recent falls. As expected, the new measures have generally been higher in the HRA of England and in Wales compared with most other countries/areas during the study period. In recent years there has been a downward trend in Wales, and to a lesser extent in the HRA of England, with respect to restriction frequency (specifically, the percentage of restricted herds with a short inter-episode interval, Figure 13), duration (the percentage of prolonged restrictions, Figure 16) and severity (Figure 18). In Northern Ireland, these measures are generally comparable to other countries/areas, although restriction length (Figure 15) (but not the percentage of prolonged restrictions, Figure 16) is increased, possibly impacted by a large proportion of small herds in Northern Ireland. Wales also has a relatively high restriction length (Figure 15), increased in recent years, and is likely associated with many bTB episodes without observed lesions since 2012 that have been subject to the same and increased number of control tests as lesioned incidents, due to a perceived heightened epidemiological risk. In the HRA of England and in Wales and Northern Ireland, there has been a recent fall in the percentage of persistent incidents (Figure 16, percentage of restrictions > 550 days), coincident with a fall in the HRA of England and in Wales of restriction severity (Figure 18, the mean number of reactors and abattoir cases per restriction). The results in Figure 16 may in part reflect differences in the average herd size in GB compared to the island of Ireland,[7] noting the recognised association between herd size and bTB persistence.[16] This is also illustrated by the change that occurs in incidence in Northern Ireland relative to other countries/areas when herd size is controlled (Figure 5, standardised annual herd incident) compared to when it is not (Figure 6, annual herd true incidence rate). In Scotland and the LRA of England, the mean number of restrictions per study herd (Figure 11) is very low, and the median restriction length for restricted herds (Figure 15) is lower than other countries/areas. Due to small numbers, the results for Scotland in several figures (Figures 13, 15, 18) need to be interpreted with care.

Policy impact is of acute interest in each of these five countries, specifically the policy/ies that have led to the greatest positive impact on bTB control. However, it is extremely difficult to attribute specific policy decisions to an observed bTB trend, due both to the complexity and fluidity of the policy mix in each of the national programmes. In other words, bTB policy and impact on bTB trends have been very difficult to disentangle. As highlighted in Table S1 (online supplementary material), there are ongoing changes to bTB policy in each of the five countries. Further, the impact of any specific policy may vary in different countries as a consequence of multiple factors, including those relating to farming (for example, livestock movement, animal housing and grazing) and the environment (*M. bovis* survival, badger density).

Throughout the five countries, those bTB policies that relate to cattle are generally quite similar, whereas those relating to wildlife are not. This reflects the focus of the relevant European legislation, principally 64/432/EEC[5] and 78/52/EEC,[6] which is prescriptive with respect to controls on cattle but essentially silent on those relating to wildlife. Key areas of difference with cattle controls include implementation of risk-based routine surveillance in Scotland, and of bTB risk areas in England (this concept has been extended to Wales since October 2017).[17] In addition, there have been differences in the use of the interferon-γ test (Table S1, online supplementary material) as there is scope within 64/432/EEC[5] for each country to decide how they use the test to best suit local conditions. Introduction and increased use of the interferon-γ test are each likely to be followed by an increase in the detection and removal of reactors (Figures 18 and 19). Wales used different interpretation thresholds for field surveillance under severe interpretation by including the additional result "severe inconclusive reactor" which would be classed as "clear testing" in the rest of the UK and which can trigger gamma testing and animal removal. Throughout the UK and Ireland, there is general agreement on the role of Eurasian badger (*Meles meles*) as a maintenance host of *M. bovis* and a contributor to the persistence of bTB in cattle, although in Scotland and the low incidence areas of England there is little evidence of any significant wildlife reservoirs of infection for cattle. Further, badgers are a protected species both in Ireland and the UK and listed under the Bern

Convention. Nonetheless, there are substantial country-level differences in approaches to wildlife as part of national bTB control (Table S1, online supplementary material). In Ireland, reactive badger removal from areas of high bTB prevalence in cattle has been used for many years, but with a recent, progressive shift to vaccination. In England, emphasis has been placed on licensed industry-led culling and voluntary vaccination in areas of endemic bTB incidence (i.e. in the HRA and parts of the edge area). A badger vaccination programme has been part of a suite of bTB controls within the Intensive Action Area (IAA) of Wales, areas where herd bTB prevalence has fallen by 35% between 2010 and 2016.[18] In Northern Ireland, badger vaccination or culling are not current policy, rather there has been a focus on evidence-gathering through a 'Test and Vaccinate or Remove' (TVR) wildlife intervention research project, a long-standing Road Traffic Accident (RTA) survey and comparison of genotype data from cattle and badger *M. bovis* positive cultures. In Scotland, routine wildlife surveillance is limited to the investigation and culture of any suspect cases in wild deer. These differing approaches to limiting infection from badgers may contribute to the observed differences in bTB trends across countries, however, this remains a point of contention.[11 19 20] Nonetheless, there is the potential for increasing convergence into the future, particularly with respect to badger vaccination. There has been substantial progress towards a field vaccination for badgers, by collaborating research groups in the UK and Ireland,[21 22] and results from the Kilkenny badger vaccine trial have been encouraging.[23 24] Oral badger vaccination against *M. bovis* infection is still at the research stage, including the formulation of a suitable oral bait, efficacy and safety studies, field deployment studies and compilation of an evidence dossier in support of a marketing authorisation for the oral vaccine.

One of the most striking results, noted previously,[7] relates to the percentage of bTB restrictions detected by abattoir surveillance, being much higher in Ireland (28.9% in 2015) than in other countries (for example, 14.9% in the HRA of England and 8.3% in Wales in 2015, Figure 8). Field and abattoir surveillance are used in each national eradication programme, with abattoir surveillance of particular importance where active testing intervals are higher, as in Scotland and the LRA of England. Given their complementary roles, country-level differences could possibly be due to country-level differences in detection sensitivity (for example, the Irish results could be due to higher sensitivity of abattoir surveillance and/or lower sensitivity for field surveillance). As explained below, however, evidence in support remains incomplete.

With respect to abattoir surveillance, there is evidence of country-level differences in detection sensitivity. In published studies investigating the effectiveness of abattoir surveillance, submission rates were substantially lower in England as a whole (1.4 to 4.1 lesions submitted per 10,000 animals killed, in 2003 and 2008 respectively),[25] compared to Ireland (22 per 10,000 in 2003-04,[26] 25 per 10,000 in 2005-07)[27] and Northern Ireland (29 per 10,000 in 2011-13).[28] In this context, submission refers to the submission of material from animals presenting with bTB-like lesions at routine slaughter. In contrast, confirmation rates were very similar across these three countries (67.4% in England during 2003-08,[25] 63.9% in Ireland during 2003-04,[26] 64.2% in Northern Ireland during 2011-13).[28] This comparison does not control for bTB prevalence among slaughtered cattle, but may in part reflect country-level differences in detection sensitivity during abattoir surveillance. Recent work has estimated the herd-level detection sensitivity during meat inspection in Irish abattoirs,[29] which at 24% would indicate that a substantial number of infected herds are not currently being detected during this procedure.

With respect to field surveillance, no direct country-level comparisons have yet been made. In all five countries, the same paired avian and bovine tuberculins from Lelystad (The Netherlands) have been used since October 2009, which is important given observed differences in test performance between tuberculin PPD combinations from different manufacturers and potencies.[30 31] Prior to October 2009, Downs and others[32] found that the use of Weybridge tuberculin was associated with slightly higher test sensitivity and lower test specificity, compared with Lelystad. During the period of interest, there have been changes to aspects of field surveillance in several countries, each with the potential to influence the sensitivity of the tuberculin skin testing regime. For example, interferon-γ testing during restrictions may lead to the identification of infected animals that may otherwise have become abattoir cases after restrictions are lifted. Further, Duignan and others[33] outline quality control measures within the Irish programme, including the ongoing assessment of tuberculin potency in cattle, and the introduction in 2010 of a means to routinely assess and rank individual testers against measures of administrative and field performance. These Irish measures may possibly have contributed to the observed fall in recent years of the percentage of bTB restrictions detected by abattoir surveillance (Figure 8). In Northern Ireland since September 2009, at least one Veterinary Officer from Standards and Compliance Branch has attended each skin test supervision to ensure a consistent approach to the audit on farm and to quality assure reports submitted to bTB programme managers. A review of bTB test audit was undertaken in 2015 and one of the early outcomes was a revised procedure at bTB test supervisions. Similarly, there have been substantial changes to the processes of approval and auditing of skin testers across GB. In 2013, APHA launched a new field audit system for official veterinarians (OVs), private veterinarians who undertake the majority of skin tests. Subsequently, an enhanced OV auditing programme has been developed and implemented by APHA to deliver a more robust quality assurance of veterinary training and skills on bTB to supplement existing training. In 2015, bTB testing in England and Wales came under a new contractual framework, so that virtually all skin testing is undertaken by regional suppliers (Delivery Partners) responsible for allocating tests to OVs and ensuring that testing is carried out to a high standard. Each Delivery Partner provides a managed service of OV testers, which includes an internal auditing system in addition to the unannounced on-farm inspection visit carried out by APHA auditors.

Divergence with respect to the percentage of herds positive at the 6-month post-restriction test is highlighted in Figure 9, being highest in the HRA of England (17.8% in 2015) and Northern Ireland (13.4%). Herd recurrence is an important feature of bTB persistence, which can be attributed either to residual infection in cattle (that is, cattle infected but missed during testing) or re-infection, either from local sources (such as spread from the environment, wildlife or neighbouring farms) or following cattle introduction. A detailed review of bTB persistence is available,[12] noting that there will be regional differences in the relative importance of these infection sources. Based on modelling work reported by Conlan and others,[34] up to 21% of herds in the HRA of England may be harboring at least one infected animal after they clear restrictions. Similarly, Clegg and others[35] confirm the importance of previous bTb exposure as a significant risk factor for large bTB breakdowns in Ireland. Abernethy and others[7] previously highlighted the challenges faced with current EU legislation, where herds are free to trade once two clear tests are achieved, that is, a minimum 4 months after the last test positive animals has been disclosed. In contrast, in the successful Australian bTB eradication programme, infected herds did not gain confirmed free 3 (CF3) status until 8 years after the last known infected animal had been removed.[36] In Ireland, Gallagher and others describe a significant improvement in herd recurrence between 1998 and 2008.[9] In Scotland, where recurrent disease has not been a feature, under 2% of OTFW episodes can be ascribed to a failure to completely eradicate a specific genotype on the first occasion, but disease has never recurred more than once (unpublished data). In addition, routine six monthly post breakdown testing was dropped in 2010 in Scotland, following a retrospective analysis of ten years worth of data showing that no confirmed reactors were found in that period at the six month post breakdown test.

c. Methodological challenges

As with the previous work,[7] we faced a number of methodological challenges during this work. Many of these relate to differences either between the national programmes or the national programme databases. The glossary of terms (Table 1) and measures of restriction frequency, duration and severity (Table 2), including the relevant study population, study period and restriction(s) of interest, were developed to ensure equivalence across the five countries.

As illustrated in Figure S4, there are differences between the study populations that were used for the existing measures and for the new descriptive measures calculating restriction frequency, duration and severity. The former existing measures relate to herds registered as active on the 1st of January in the year of interest (2014 in the case of Figure S4(a)) and a subset of these herds that yielded a bTB test in a rolling period 2 years prior to and after this date (2012-2015, Figure S4(b). In contrast, the latter new descriptive measures represent the cohort of herds that were present throughout the full study period, 2003-2015 (specifically, presented for testing in each of the following periods: 2002-05 (2001 England, Scotland and Wales due to a Foot & Mouth Disease outbreak), 2006-10, 2011-15, Figure S4(c)). This selection criteria would exclude herds in areas with long test intervals or with test-exempt status. Herds that were depopulated, changed ownership, changed registration number, went dormant and reactivated or newly registered herds would all be lost from the study population. These differences are greatest along the eastern half of England and Scotland, which is most likely to be a product of testing every four years instead of annually which is mostly the case elsewhere. A herd without cattle eligible for testing at its scheduled test in a four yearly testing area cannot qualify for the study cohort through this test and it may be four years until its next test, whereas in an annually tested area the next scheduled test will be in just one year’s time. It is thus far more likely for a four yearly tested herd not to be tested in one of the five-year study periods, and throughout the study period this would include areas of varying sizes in Western England and Wales too. Around 21% of herds contributing to existing measures in 2014 in GB were not formed until after 2005 and thus could not qualify in the new measures study cohort. The percentage herd loss per hexagon shown in Figure S5 will include such herds in calculating this loss.

Some difficulties were faced when seeking equivalence with respect to the start and end of bTB restrictions. In Northern Ireland, for example, the period of restriction is initiated on the date that a herd loses its OTF status. However, the initiation date does not correspond exactly with the bTB disclosure date for those restrictions where the test date has been delayed. After considering all restricted periods during 2010-2015, this equates to a duration increase of 2.92 days (95% CI: 2.87-2.97) per restriction; therefore, the impact of this discrepancy is minor (but does contribute to the higher trend line for Northern Ireland in Figure 15). In GB, restrictions are lifted by the service of the TB10 notice, but this can be weeks or months after the final control test, which in Ireland and Northern Ireland is typically when restrictions are lifted. Adjustments were therefore made to the restriction end date in GB so that they equated to that final control test in order to replicate as far as possible the situation in Ireland and NI. 5% could not be confidently adjusted and the official TB10 date retained. In the study, herd incidence density rates were introduced, to account for herd-years at-risk. Equivalent methods have been used to allow comparison between countries. However, no adjustment is made for herd size, and therefore comparison between GB and the island of Ireland is difficult as the latter is characterised by smaller herds.[7] Further, direct comparison with current published GB figures on herd incidence density rates is not possible due to differing methodologies (the denominator here accounts for the number of days unrestricted during the year of interest; in the current published official GB figures,[37] account is taken of the number of days from the start of restriction back to the last measure of a herd's bTB free status, which would be either the preceding clear whole herd test or the end of the previous restrictions). This was necessary due to differences between countries in data management and interpretation. To account for country-level differences, left censoring was applied (Table 2), therefore care is needed when interpreting measures of restriction duration earlier in the study period.

d. Conclusions, recommendations for further research

In conclusion, we have presented a further description of bTB trends in the UK and Ireland, during the period from 2003 to 2015, building on earlier work.[1] This work is unique in that it uses a common framework across five countries, and will assist policy-makers when critically evaluating policy options for effective control and the progress of eradication programmes. Ongoing updates would be useful, potentially every 5 years, providing an evidence base for country-level comparison of bTB trends into the future. There is a need to critically evaluate the usefulness of existing statistical measures to describe the progress of bTB, and to introduce and evaluate new concepts as they become available, such as whole genome sequencing. The use of multivariable analytical methods should be considered, but will rely on substantial sharing of raw data across the five countries, as was achieved here in support of the spatial analyses. Spatial and temporal trends could be explored using a range of methodologies, including analytical techniques in GIS, to evaluate trends and relative changes in bTB prevalence.[38]

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Chapter 6

Northern Ireland Farm Level Management Factors for Prolonged Bovine Tuberculosis Herd Breakdowns

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Original Paper

Northern Ireland Farm Level Management Factors for Prolonged Bovine Tuberculosis Herd Breakdowns

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Running head: Bovine tuberculosis surveillance

SUMMARY

This study determined farm management factors associated to long duration bTB (bovine Tuberculosis) breakdowns disclosed in the period 23/05/2016 to 21/05/2018; a study area not previously subject to investigation in Northern Ireland. A farm level epidemiological investigation (n=2935) was completed when one or more SICCT (Single Intradermal Comparative Cervical Test) reactors or when one or more confirmed (positive histological and/or bacteriological result) LRS (lesion at routine slaughter) were disclosed. A case control study design was used to construct an explanatory set of management factors associated to long duration bTB herd breakdowns; with a case (n=191) defined as an investigation into a breakdown of 365 days or longer. Purchase of infected animal(s) had the strongest association as the most likely source of infection for long duration bTB herd breakdowns followed by badgers and then cattle-to-cattle contiguous herd spread. However, 73.5% (95% CI: 61.1-85.9%) of the herd type contributing to the purchase of infection source were defined as beef fattening herds. This result demonstrates two subpopulations of prolonged bTB breakdowns, the first being beef fattening herds with main source continuous purchase of infected animals and a second group of primary production herds (dairy, beef cows and mixed) with risk from multiple sources.

Key words: Epidemiology, *Mycobacterium bovis,* bovine tuberculosis, Case-Control Study, Chronic breakdowns, Cattle.

INTRODUCTION

Bovine tuberculosis (bTB) caused by *Mycobacterium bovis is* a zoonotic disease primarily affecting animals. Although cattle are the main hosts, the disease has been reported in many other farmed and wild animals[1]. DAERA (Department of Agriculture, Environment and Rural Affairs) has an EU (European Union) Commission approved bTB eradication programme which ensures compliance with the EU Trade Directive 64/432/EEC. EU approval of the bTB Northern Ireland eradication programme is vital in safeguarding the export dependent livestock and livestock products industry (worth in excess of £1.79 billion in 2018)[2]. In 2018 the Northern Ireland bTB programme cost £39 million, an increase of £8.5 million from 2016. This increase was reflective of increased disease incidence from 2016 to 2017 (herd incidence of 9.61% in December 2017 compared to 7.45% in December 2016) requiring associated increased expenditure largely in the area of compensation payment for the purchase of cattle as part of the bTB programme[3].

In 2016, a bTB eradication strategy for Northern Ireland was published [4], providing a framework for bTB eradication from the national cattle population. Part of the implementation plan for this strategy recommended that herds chronically infected with bTB ('chronic herds') should be recognised as a distinct entity for action and a package of measures be targeted at them so as to minimise their impact.

In a previous publication [5], data from a national database APHIS (Animal and Public Health Information System)[6] was used to determine definitions for chronic herds. The definitions developed for both long duration and recurrent bTB herd breakdowns encompassed almost 40% of the total number of Single Intradermal Comparative Tuberculin Test (SICCT) reactors identified during the study period [5]. This study looked at risk factors pertaining to the SICCT and cattle movement data stored on APHIS, implementing a design (same design as our present study) which compared prolonged bTB breakdowns to short duration breakdowns [5]. However, the original study [5] could not investigate any of the bTB herd breakdown risk factors associated with chronic herds at a farm management level, a knowledge gap in Northern Ireland which our present study aimed to fill. Such management factors have been studied in Great Britain and the Republic of Ireland [7, 8]. A previous case control study in Northern Ireland considered risk factors for bTB relating to farm boundaries, neighbouring herds and wildlife, but it did not investigate chronic bTB herd breakdowns[9] and in a design contrast to our study it compared herds with breakdowns to herds which did not experience bTB breakdowns.

Study Objective

The objective of this study was to identify farm level management factors associated with prolonged bTB herd breakdowns, using data collected during on-farm epidemiological investigations. A case-control study design was used where prolonged duration bTB herd breakdowns (as defined previously [5]) were compared to short duration bTB herd breakdowns.

MATERIALS AND METHODS

Study design and data collection

A case control study was conducted on a study population consisting of all bTB herd breakdown investigations during the period 23/05/2016 to 21/05/2018. Data collection involved completion of an on-farm investigation form when one or more SICCT reactors or one or more confirmed LRS (Lesion at routine slaughter) were disclosed in any Northern Ireland cattle herd. Confirmation of bTB in an LRS was defined as a positive histological and/or bacteriological culture result following laboratory examination. Investigations were carried out by trained Animal Health and Welfare Inspectors (AHWI) who visited each of the bTB breakdown farms. At each farm, an on-site questionnaire was completed (supplementary table S2) through face-to-face interview of the farmer, including identification of all herds contiguous to the bTB herd breakdown. Based on the completed questionnaire and local knowledge of the area, the VO (Veterinary Officer) responsible for the bTB herd breakdown, where possible, determined the most likely source of infection for the breakdown. Questionnaire information along with data extracted from APHIS (herd size and location) were collated into Microsoft Access^{TM} (Microsoft Corporation, Redmond, WA, USA). For our study, the ten Divisional Veterinary Offices (DVOs) were aggregated into three groups according to their geographic location; south east group (Armagh, Newry, Newtownards), west group (Dungannon, Enniskillen, Strabane, Omagh) and north east group (Ballymena, Coleraine, Mallusk) (Figure 1).

Case and control definitions were identical to those used in a previous study [5]. Cases were bTB herd breakdowns which ended during the study period (23/05/2016 to 21/05/2018) and had a duration of greater than or equal to 365 days. Controls were bTB herd breakdowns which ended during the study period (23/05/2016 to 21/05/2018) and had a duration of less than 365 days.

Data analysis

Microsoft Access^{TM} (Microsoft Corporation, Redmond, WA, USA) and R Version 3.4.0[3] were used for data manipulations and R Version 3.4.0[a] and Stata/SE 15[4] were used for data analysis. The model framework used was binary logistic regression using purposeful selection of covariates [10] with the case definition forming the response variable. In total 78 explanatory variables were derived from the on-farm questionnaire (see supplementary table S2) along with their associated factor levels. Initially all variables were tabulated using the duration case definition against each variable's factor levels. As variables were added or removed from the model, the AIC (Akaike Information Criterion) difference was calculated between the old and new proposed model, in order to determine if the proposal reduced AIC by a value greater than two [10]. Where the models were subsets of each other, the LRT (Likelihood Ratio test) was also calculated in order to determine if addition or removal of variables was significant at the $P \leq 0.05$ level.

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[3] R Core Team (2017). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL https://www.R-project.org/.

[4] StataCorp. 2017. Stata Statistical Software: Release 15. College Station, TX: StataCorp LP.

Initial analysis was by univariable logistic regression. Any variables containing low numbers (<10) of cases at any factor level, which could not have that factor level logically merged with another level were removed after univariable analysis. Remaining variables with $P \leq 0.25$ were then analysed using a multivariable logistic regression model. The resultant model was further refined to produce a reduced multivariable logistic regression model which utilised variables with $P \leq 0.05$ from the first multivariable model. Following the fit of the reduced multivariable model its estimated coefficients were compared to those in the initial multivariable model to determine if there was a magnitude change of > 20%. This magnitude change known as $\Delta\hat{\beta}\%$ (Delta-Beta-Hat %) indicates that one or more of the excluded variables are important in the sense of providing a needed adjustment effect of the variables that remained in the model [10]. Variables which formed the first multivariable model but not included in the initial reduced multivariable model were added back individually; being retained if they contributed to the overall model and reduced $\Delta\hat{\beta}\%$ to below 20%.

Further to this, variables with $P > 0.25$ in the initial univariable analysis were also individually added back in to determine if they contributed to the multivariable model thus producing the preliminary main effects model. The only continuous variable included in the preliminary main effects model was herd size. Fractional polynomial analysis[11] was applied to herd size in order to determine if it required scale transformation so as to satisfy the assumption of linearity in the logit outcome. Completion of this stage produced the main effects model. Using the variables present in the main effects model all combinations of two way interactions were statistically assessed using the LRT ($P < 0.05$); however, only those with probable clinical significance were accepted as potential candidates for the model. Interaction terms accepted into the final model had an odds ratio calculated as a linear combination with their associated main effects ($\beta_0 + \beta_1 + \beta_2 + \beta_1.\beta_2$) and the results placed into Table 3. The finalized model was then subjected to the Hosmer-Lemeshow goodness-of-fit-test (decile sub-grouped) to determine how well it fitted the data. Table 1 details how the methodology was applied in this study to achieve the final multivariable model.

RESULTS

A total of 2935 bTB herd breakdown investigations were completed during the study period. Supplementary table S1 provides summary details of the 78 study variables for cases and controls. There were 126 ongoing bTB herd breakdowns at the end of the study period which were removed, leaving 2809 valid bTB herd breakdowns (191 cases and 2618 controls; Supplementary table S1). Table 1 details the results returned at each stage of the model building process from univariable analysis through to final multivariable model. As a result of carrying out fractional polynomial analysis [11] on herd size it was transformed to herd size to power 0.5 and was then referred to as 'herd size transformed' in the subsequent analysis.

The results from the final model (Table 2) demonstrated that the odds ratio of a bTB herd breakdown persisting greater than 365 days that contained pedigree animals was 0.594 (95%CI: 0.402-0.863); where fluke treatment was carried out on the farm was 0.263 (95%CI: 0.139-0.528), where cattle can access grazing ground to which slurry has been freshly applied was 0.525 (95%CI: 0.283-0.915); where there was partial upgrading of boundary fences in the last three years was 0.383 (95% CI: 0.247-0.588); where there was full upgrade of boundary fences in the last three years was 0.599 (95% CI: 0.406-0.886); where dead badgers were found on roads within 1.6 km of the farm in the past three years was 1.810 (95% CI: 1.268-2.616); manure spread on grazing ground was 1.289 (95% CI: 0.926-1.798); use of IBR vaccination on the farm was 1.476 (95% CI: 1.005-2.158) and use of leptospirosis vaccination on the farm was 0.631 (95% CI: 0.391-0.999). Dairy herds accounted for 73.2% (95% CI: 59.6-86.7%) of bTB herd breakdown which carry out leptospirosis vaccination. The presence of a badger sett was recorded on 29.87% (95% CI: 28.18-31.56) of investigations and of these 2.95% (95% CI: 2.33-3.58%) reported fencing off badger setts and latrines.

The results of the linear combination of the five two way interaction terms added to the model and their associated main effects ($\beta_0 + \beta_1 + \beta_2 + \beta_1.\beta_2$) are shown in Table 3. Of the five two way interaction terms added to the main effects model (Table 3), 'mixed grazing of cattle and sheep' x 'do cattle drink from natural sources of water' and 'DVO of the bTB breakdown' x 'woodland on the farm or within 1.6km of the farm' returned odds ratios not significantly different from one, when interpreted as a linear combination with the main effects.

With the other three interaction terms, 'DVO of the bTB breakdown' x 'herd size transformed', 'bTB breakdown risk picked as most likely source by VO' x 'herd size transformed' and 'purchase of store cattle at a market within previous five years' x 'herd size transformed' have their results from Table 3 shown in figures 2, 3 and 4, respectively, so that these can be interpreted in association with increasing herd size (untransformed). The odds ratio of a case where a farm is located in DVO south east group and there is an increase in herd size transformed by one was 0.968 (95%CI: 0.895-1.047), with DVO west group and herd size transformed increase by one was 1.005 (95%CI: 0.907-1.132) and with DVO north east group and herd size transformed increase by one was 1.069 (95%CI: 0.899-1.124).

Figure 2 shows the effect on the duration case definition as herd size increases in each of the three DVO groups. The variables most likely risk source and herd size transformed had a significant interaction on addition to the main effects model (LRT: P=0.001). The odds ratio of a case for the base line group and where there is an increase in herd size transformed by one had was 0.968 (95%CI: 0.895-1.047), with cattle to cattle contiguous herd spread of infection source and herd size transformed increase by one was 1.046 (95%CI: 0.993-1.173), with purchase of infected animal(s) source and herd size transformed increase by one was 1.119 (95%CI: 0.993-1.261), with carryover of infection source and herd size transformed increase by one was 1.002 (95%CI: 0.865-1.161) and with badger infection source and herd size transformed increase by one was 1.071 (95%CI: 0.954-1.203).

Figure 3 shows the effect on the duration case definition of increasing herd size in each of the five infection source groups. Looking at herd types for the duration case definition based on whether they are beef fattening or other (dairy, beef cow or mixed) 11.4% (95% CI: 1.9-20.7%) of beef fattening herds have source of infection as cattle to cattle spread, 73.5% (95% CI: 61.1-85.9%) have purchase of infection as source, 0% for carryover of infection and 12.8% (95% CI: 3.2-22.3%) have a badger source of infection. Variables purchase of store cattle at a market in the previous five years and herd size transformed had a significant interaction on addition to the main effects model (LRT: P<0.001). The odds ratio of a case where a farm purchases store cattle from a cattle market, there was an increase in herd size transformed by one was 1.050 (95%CI: 0.950-1.160). Figure 4 shows the effect on the duration case definition of increasing herd size for purchase of store cattle versus non-purchase.

Discussion

One of the key perspectives of this work is to further elucidate chronic long duration bTB herd breakdowns (>365 days) through provision of a quantitative characterisation of their infection source thus facilitating formulation of a more focused disease control policy. Cattle movement has been identified as a consistent herd level risk factor for bTB [12]. In our study, two cattle movement factors were statistically associated with long duration bTB herd breakdowns, namely, purchase of cattle generally and specifically purchase of store cattle (calves purchased for feeding over winter). Both of these factors had a significant interaction with herd size (Fig 4), which demonstrated that given purchase of cattle risk factors, larger herds were more likely to have prolonged bTB herd breakdowns.

Cattle purchase had the strongest association as the most likely infection source (Fig 3) followed by wildlife (badgers) and then contiguous herd spread (cattle to cattle). However, when selectively looking at prolonged bTB herd breakdowns, three-quarters (73.5%) of the herd type contributing to the purchase of infection source were beef fattening herds. This highlights two subpopulations of long duration bTB herd breakdowns, the first being beef fattening herds which have long duration breakdowns due to continuous purchase of infected animals and a second group of primary production herds (dairy, beef cows and mixed) with long duration breakdowns due to infection risk from multiple sources.

After purchase of infection, badgers formed the next most likely source of a long duration bTB herd breakdown, which was a risk factor for bTB breakdowns identified in other studies [13-16]. Skuce et al. (2012) generalize the risk from badgers to indicators of badger density/activity[12] and, in terms of chronic bTB herd breakdowns in Republic of Ireland (ROI), badger presence was reported as a risk factor for dairy herds[7]. The findings from our study is consistent with these previous studies, with the association with prolonged bTB herd breakdowns being further affirmed by the finding of dead badgers on a road <1.6kms from the home farm.

A VO attributing badgers as the most likely infection source also had significant interaction with herd size (Fig 3) which showed this source in larger herds increased the odds of a prolonged bTB herd breakdown. This is not surprising as large herds tend to require a larger grassland area for feeding purposes, which increases the probability of exposure to a larger number of badgers, which is compounded by the larger number of cattle in such herds.

Two badger related variables excluded from the model due to low case numbers were fencing off of badger setts and fencing off of badger latrines; both of which are considered preventive measures. An important observation in this study was that almost one-third (30%) of farms were observed as having badger setts, but only 3% of investigations reported farms where badger setts and/or latrines were fenced off, an observation also reported in other Northern Irish work [14]. Without extensive fencing off of badger setts and latrines indirect contact between badgers and cattle cannot be curtailed[17].

The third important source of infection was cattle-to-cattle contiguous herd spread. Previously, Denny and Wilesmith (1999) reported that in Northern Ireland the two main associations with bTB breakdowns were the presence of badgers and contiguous neighbours who had confirmed bTB breakdowns (aetiological fraction for both was approximately 40% each)[9]. They also stated that 79% of fences in Northern Ireland did not prevent nose to nose contact between herds[9]. In a more recent Northern Ireland study, contact between neighbouring cattle was assessed as possible through 66.8% of boundaries, however no significant association was found between boundary contact and bTB breakdown[14]. Our study, which looked specifically at prolonged bTB herd breakdowns, found that both recent upgrading or complete installation of new boundary fences showed significant negative association with duration of bTB breakdowns (OR = 0.383: 95% CI 0.247-0.588 and OR = 0.599: 95% CI 0.406-0.886, respectively). This result provides circumstantial evidence for the application of better biosecurity measures in the form of adequate boundary fences to reduce cattle-to-cattle contiguous herd contact could reduce the odds of a prolonged bTB herd breakdown.

This study also investigated associations with other common diseases found on Northern Ireland farms. Application of fluke treatment was significantly associated with a reduced odd of developing a prolonged bTB herd breakdown. Co-infection with liver fluke may mask the true bTB infection status of animals making SICCT clearance of the herd difficult [18]. Indeed, given the widespread prevalence and high level of press coverage relating to fluke infection (and its potential link to bTB) it is surprising that not more herd keepers treat their cattle against liver fluke.

Other disease related variables investigated were use of IBR (Infectious Bovine Rhinotracheitis) vaccination and Leptospirosis vaccination. Skuce et al. (2012) stated that the influence of respiratory infections on the susceptibility to infection with *M. bovis* remains untested but speculated that such infections can facilitate increased aerosol spread[12]. Our study showed an association between use of IBR vaccination (used as a proxy for IBR exposure within a herd) and increased odds of developing a prolonged breakdown (OR = 1.476). With Leptospirosis vaccination, there was a negative association between its use and development of a prolonged bTB herd breakdown (OR = 0.631), although the significance was marginal (P=0.054) and given that 73% of these prolonged bTB herd breakdowns were in dairy herds may suggest that other management factors confound this finding.

Presence of registered pedigree animals on a farm was significantly associated with a reduced odds of developing a prolonged bTB herd breakdown. Association with this variable indicated that herds containing pedigree animals appear to be better at removing infection. Presence of pedigree animals on a farm is probably indicative of a herd where trade and movement are important to the business, thus providing very strong motivation for a farmer to clear infection from the herd and employ improved biosecurity measures.

Several studies have looked at the area of risk presented by slurry and manure derived from bTB infected premises; but definitive results on the subject are few. Given the type of cases in our study and suggestions that approximately six months are required for deactivation of *M. bovis* in contaminated slurry [19], they must represent an extreme in terms of potential for production of bTB infected slurry or manure. The variable "manure spread on grazing ground" had a positive association to prolonged bTB herd breakdowns (however not statistically significant in the final model, odds ratio = 1.289 (95% CI: 0.926-1.798), a result consistent with previous work [19]), but was included as it provided adjustment effects for other variables. Our study indicated no association with the use of contractors for slurry spreading (at odds with another Northern Irish study [14]) or with applying slurry/manure to grazing ground. Even in situations where cattle can access ground to which fresh slurry has been applied, the results point to a negative association (OR = 0.525) with cases, However, given the low number of cases (n=17) in this category, it would require a guarded interpretation. The results show that herds located in the DVO north east group have the strongest statistical association to the prolonged breakdown case definition. Additionally there is an interaction between DVO herd group and herd size (Fig 2) where relative to the others, odds of a prolonged bTB herd breakdown increase for north east herds with increasing herd size.

Given that in this study a statistical significance cut off level of P<0.05 was used for variable selection and that the final multivariable model contained 16 variables it should be realised that inclusion of at least one spurious association is a possibility. It is also possible with a study design where VO's select a breakdown source of infection there is potential for a degree of subjectivity. However, it is the trained VO with their local knowledge and standardised guidance who are best placed to make these assessments.

CONCLUSIONS

One of the central tenets of this work was investigation of disease source and its relationship to long duration bTB herd breakdowns. The source with the strongest association to long duration breakdowns was purchase of infection; however, as a source, it applies mainly to beef fattening herds. Beef fattening herds mostly move their stock to an abattoir with very few cattle movements to other herds. However, they do present a risk from the continuous output of infection to local wildlife and to other herds grazed contiguously. In order to reduce the input of infection to these herds they must have the capability to risk assess their purchases [20] thus reducing their overall ability to act as an infection focus in their locality. With herds other than beef fattening herds, the source of infection for long duration breakdowns are multiple and must be addressed in a multi-faceted way.

In terms of wildlife source, more effort must be placed into breaking the transmission links between cattle and badgers. This could involve an array of methods varying from those directly applied to the badgers through to methodical and efficient fencing of badger setts and latrines. Indeed this work shows that basic segregation methods to separate badgers and cattle using fencing are not being applied. The low levels of fencing off by farmers could be as a result of confused communication, occurring where one section of government responsible for bTB control promotes it as necessary, while another section implementing subsidy payments (Basic Payment Scheme) contradicts it by officially removing these fenced off areas from field maps, potentially affecting payment. It is thus essential that governments do not create contradicting messages with policy implementation and should conceive more effective ways of promoting best practice [21], such as consulting widely before introducing future subsidies, to ensure their application is biosecurity friendly and communicating this effectively to the farming public. Indeed demonstrating what is possible, some areas fenced off as part of agri-environment schemes have now been deemed eligible for BPS area based payments, a model which should be implemented in relation to farm biosecurity.

The other source shown to be linked to long duration breakdowns was cattle to cattle contiguous herd spread and without effective biosecure boundaries between herds this infection route will remain present. This again is an area where it should be possible for government to intervene, incentivising the good practice of constructing bio secure boundary fences between neighbouring farms and penalising situations where poor fencing risks contiguous disease spread. Effective boundary fencing would form a necessary part of an overall biosecurity package aimed at the structural elements of the ongoing bTB problem.

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CONFLICT OF INTEREST

None.

DATA AVAILABILITY STATEMENT The data that support the findings of this study is not publically available data.

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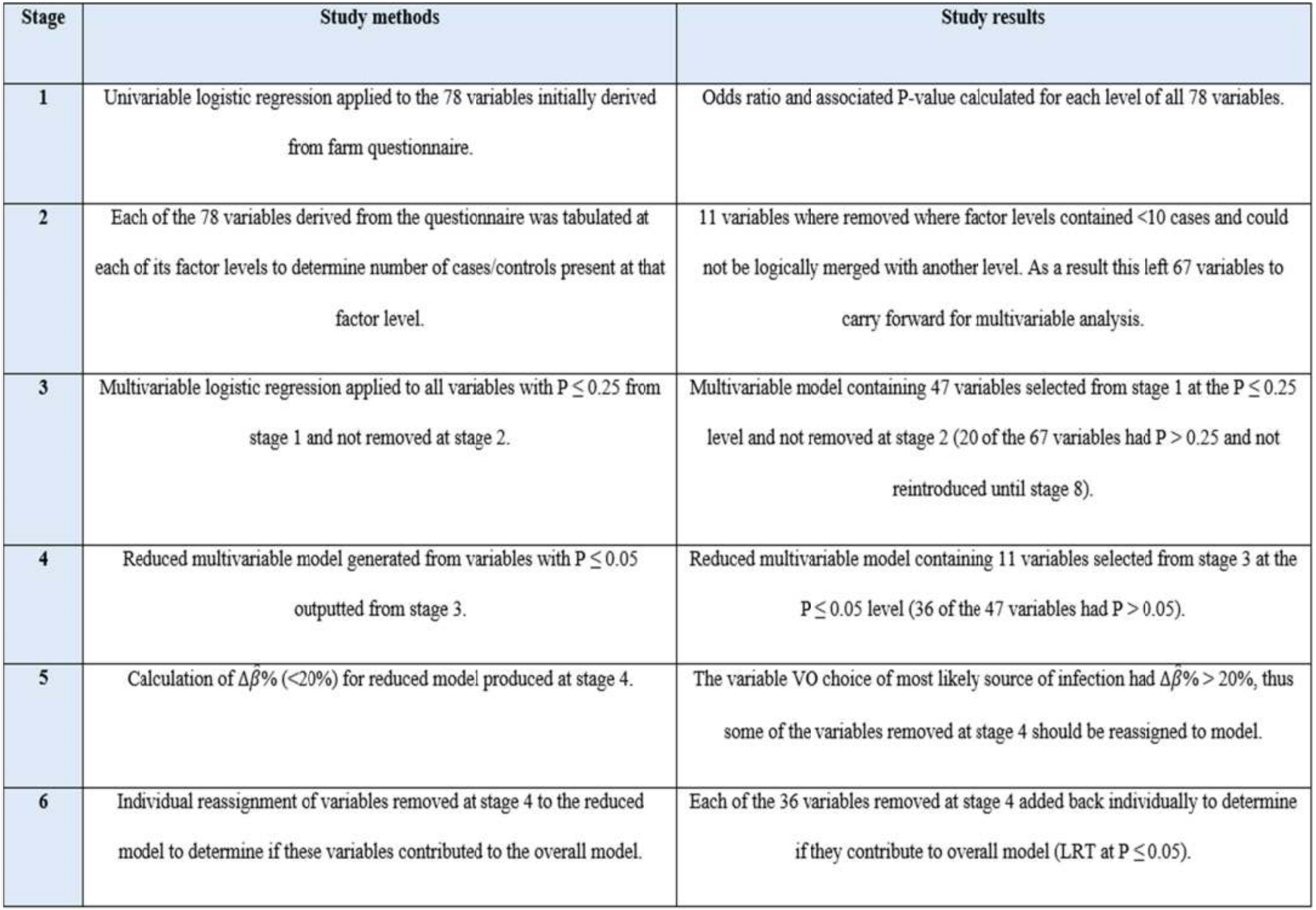
Table 1: Table showing methods applied and results observed at each stage of the study model building process.



Stage	Study methods	Study results
1	Univariable logistic regression applied to the 78 variables initially derived from farm questionnaire.	Odds ratio and associated P-value calculated for each level of all 78 variables.
2	Each of the 78 variables derived from the questionnaire was tabulated at each of its factor levels to determine number of cases/controls present at that factor level.	11 variables where removed where factor levels contained <10 cases and could not be logically merged with another level. As a result this left 67 variables to carry forward for multivariable analysis.
3	Multivariable logistic regression applied to all variables with $P \leq 0.25$ from stage 1 and not removed at stage 2.	Multivariable model containing 47 variables selected from stage 1 at the $P \leq 0.25$ level and not removed at stage 2 (20 of the 67 variables had $P > 0.25$ and not reintroduced until stage 8).
4	Reduced multivariable model generated from variables with $P \leq 0.05$ outputted from stage 3.	Reduced multivariable model containing 11 variables selected from stage 3 at the $P \leq 0.05$ level (36 of the 47 variables had $P > 0.05$).
5	Calculation of $\Delta\hat{\beta}\%$ (<20%) for reduced model produced at stage 4.	The variable VO choice of most likely source of infection had $\Delta\hat{\beta}\% > 20\%$, thus some of the variables removed at stage 4 should be reassigned to model.
6	Individual reassignment of variables removed at stage 4 to the reduced model to determine if these variables contributed to the overall model.	Each of the 36 variables removed at stage 4 added back individually to determine if they contribute to overall model (LRT at $P \leq 0.05$).

Table 1 continued

Stage	Study methods	Study results
7	Reduced multivariable model refined by variable addition/removal to obtain $\Delta\hat{\beta}\% < 20\%$ for all variables included in the multivariable model.	Refining of reduced multivariable model from stage 4 results in addition of four new variables: 1. Purchase of store cattle < five years previous. 2. Manure spread on grazing ground. 3. VO second choice source of infection 4. Herd vaccinated against IBR. All $\Delta\hat{\beta}\%$ for variables in the multivariable model at this stage of model building were <20%.
8	Individual reassignment of variables removed at stage 3 to the model produced at stage 7 to determine if these variables contributed to the overall model. Output from this stage formed the preliminary main effects model.	Each of the 20 variables removed at stage 3 added back individually to determine if they contribute to the overall model (LRT at $P \leq 0.05$). Addition of the variable – Herd vaccination against Leptospirosis gives the preliminary main effects model.
9	Fractional polynomial analysis to assess linearity of any continuous variables to the outcome.	Analysis results in the variable transformation of herd size to a power of 0.5. Herd size is added in its transformed state to the preliminary model to form the main effects model and was referred to as herd size transformed.
10	All combinations of two way interactions (LRT at $P \leq 0.05$ and judged as clinically significant) from main effects model were added to main effects model. Interactions retained or removed based on p-value ($P \leq 0.05$) within	Five interaction terms were added to the main effects model of 16 variables to form the final model. Odds ratio for these interactions in linear combination with their main effects were calculated and added to Table 3.

Table 1 continued

Stage	Study methods	Study results
	the model and as to whether they lead to model improvement (AIC and LRT cut offs already described). These interaction variable pairs were then evaluated as a linear combination ($\beta_0 + \beta_1 + \beta_2 + \beta_1.\beta_2$) with the main effects to determine their overall odds ratio and associated confidence interval (Table 3).	1. Mixed grazing of cattle/sheep x Cattle drink from natural sources of water (LRT: P=0.015) 2. Any woodland on farm or <1.6km of the farm x DVO of the bTB breakdown (LRT: P=0.017) 3. DVO of the bTB breakdown x Herd size transformed (LRT: P=0.020) 4. bTB breakdown risk most likely source chosen by VO x Herd size transformed (LRT: P=0.001) 5. Purchase of store cattle at market <five years x Herd size transformed (LRT: P=0.001)
11	Application of the goodness of fit test (Hosmer and Lemeshow goodness of fit test) and variable correlation analysis to the final model.	Hosmer and Lemeshow goodness of fit test result: P= 0.582. Provides evidence at $P \leq 0.05$ level of adequate goodness of fit.

Table 2: Results of final multivariable case control study containing categorical and continuous variables (note interaction terms are included in table 3)

Study Variable Categorical	Exposure Level	Case: TB breakdown with duration 365 days or greater (n=191)		Control: TB breakdown with duration less than 365 days. (n=2618)		Odds Ratio	95% CI	P-Value
	(NA=Not Applicable)	n	%	n	%			
Registered pedigree animals present on the farm	No	144	7.6	1751	92.4	-	-	-
	Yes	47	5.1	867	94.9	0.594	0.402-0.863	0.007
Mixed grazing of cattle and sheep	No	139	6.3	2074	93.7	-	-	-
	Yes	52	8.7	544	91.3	2.278	1.328-3.810	0.002
Do cattle drink from natural sources of water	No	95	6.2	1443	93.8	-	-	-
	Yes	96	7.6	1175	92.4	1.655	1.136-2.416	0.009
	No	15	17.4	71	82.6	-	-	-

Table 2 continued

Study Variable Categorical	Exposure Level	Case: TB breakdown with duration 365 days or greater (n=191)		Control: TB breakdown with duration less than 365 days. (n=2618)		Odds Ratio	95% CI	P-Value
	(NA=Not Applicable)	n	%	n	%			
Fluke treatment carried out on the farm	Yes	176	6.5	2547	93.5	0.263	0.139-0.528	0.000
Cattle access to grazing ground to which slurry freshly applied	No or NA	174	7.1	2289	92.9	-	-	
	Yes	17	4.9	329	95.1	0.525	0.283-0.915	0.031
Any boundary fence with a neighbour upgraded in the past three years (where full upgrade is installation of a complete new fence)	No	63	9.2	621	90.8	-	-	-
	Some upgrading	43	4.2	982	95.8	0.383	0.247-0.588	0.000
	Full upgrading	85	7.7	1015	92.3	0.599	0.406-0.886	0.010

Table 2 continued

Study Variable Categorical	Exposure Level	Case: TB breakdown with duration 365 days or greater (n=191)		Control: TB breakdown with duration less than 365 days. (n=2618)		Odds Ratio	95% CI	P-Value
	(NA=Not Applicable)	n	%	n	%			
Any dead badgers seen on roads within 1.6km from any of your land in past three years	No	181	6.9	2462	93.1	-	-	-
	Yes	10	6.0	156	94.0	1.81	1.268-2.616	0.001
Any woodland on the farm or within 1.6km of the farm	No	93	7.8	1095	92.2	-	-	-
	Yes	98	6.1	1523	93.9	0.524	0.310-0.878	0.015
Divisional Veterinary Office of the bTB breakdown	Armagh, Newry, Newtownards	73	9.1	733	90.9	-	-	-
	Dungannon, Enniskillen, Strabane, Omagh	76	5.7	1252	94.3	0.383	0.161-0.910	0.030

Table 2 continued

Study Variable Categorical	Exposure Level	Case: TB breakdown with duration 365 days or greater (n=191)		Control: TB breakdown with duration less than 365 days. (n=2618)		Odds Ratio	95% CI	P-Value
	(NA=Not Applicable)	n	%	n	%			
	Ballymena, Coleraine, Mallusk	42	6.2	633	93.8	0.071	0.018-0.247	0.000
bTB breakdown risk picked as most likely source by VO	Source of infection not established (includes Other and Deer source)	38	4.3	844	95.7	-	-	-
	Cattle to cattle contiguous herd spread	44	7.0	589	93.0	0.495	0.166-1.447	0.203
	Purchase of infected animal(s)	49	9.9	448	90.1	0.328	0.103-1.018	0.055
	Carryover of previous infection	13	6.6	184	93.4	0.896	0.150-4.773	0.901
	Badgers	47	7.8	553	92.2	0.573	0.189-1.703	0.320

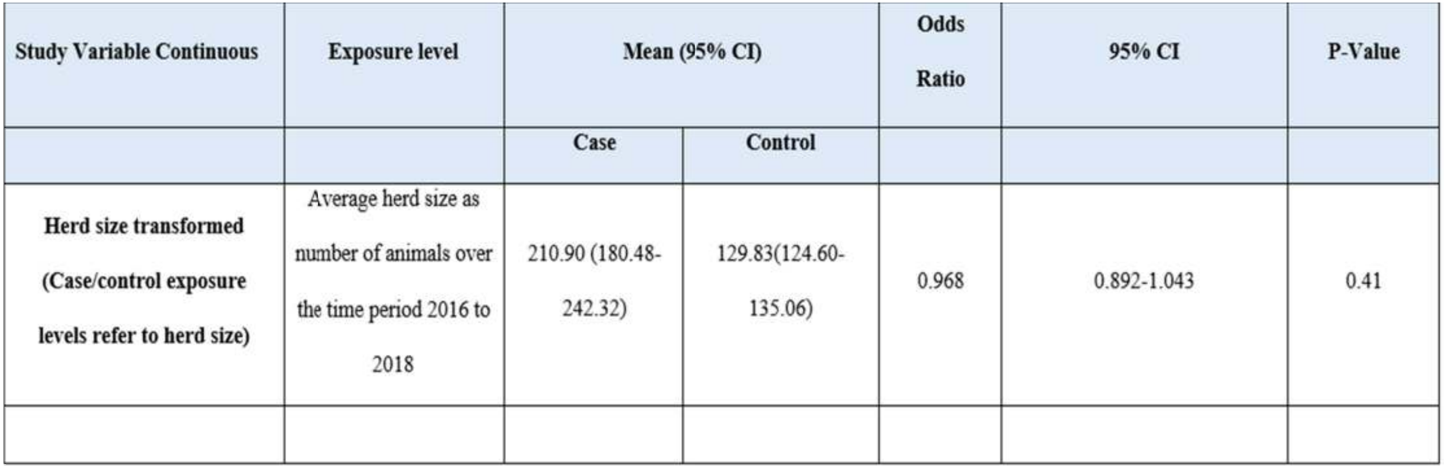
Table 2 continued

Study Variable Categorical	Exposure Level	Case: TB breakdown with duration 365 days or greater (n=191)		Control: TB breakdown with duration less than 365 days. (n=2618)		Odds Ratio	95% CI	P-Value
	(NA=Not Applicable)	n	%	n	%			
Purchase of store cattle at a market within the previous five years.	No	90	5.5	1545	94.5	-	-	-
	Yes	101	8.6	1073	91.4	0.604	0.271-1.333	0.215
Manure spread on grazing ground	No	88	5.9	1410	94.1	-	-	-
	Yes	103	7.9	1208	92.1	1.289	0.926-1.798	0.133
bTB breakdown risk picked as second most likely source by VO	Source of infection not established (includes Other and Deer source)	17	6.0	267	94.0	-	-	-
	Cattle to cattle contiguous herd spread	45	9.0	454	91.0	1.181	0.627-2.312	0.616
	Purchase of infected animal(s)	13	8.6	138	91.4	1.481	0.638-3.385	0.353

Table 2 continued

Study Variable Categorical	Exposure Level	Case: TB breakdown with duration 365 days or greater (n=191)		Control: TB breakdown with duration less than 365 days. (n=2618)		Odds Ratio	95% CI	P-Value
	(NA=Not Applicable)	n	%	n	%			
	Carryover of previous infection	12	9.5	114	90.5	1.294	0.540-3.021	0.554
	Badgers	47	7.5	577	92.5	1.503	0.778-3.022	0.237
	No second choice source selected	57	5.1	1068	94.9	0.709	0.379-1.383	0.297
Herd vaccinated against IBR	No	111	5.6	1853	94.3	-	-	-
	Yes	80	9.5	765	90.5	1.476	1.005-2.158	0.045
Herd vaccinated against Leptospirosis	No	174	6.7	2439	93.3	-	-	-
	Yes	17	8.7	179	91.3	0.631	0.391-0.999	0.054

Table 2 continued



Study Variable Continuous	**Exposure level**	**Mean (95% CI)**		**Odds Ratio**	**95% CI**	**P-Value**
		Case	**Control**			
Herd size transformed (Case/control exposure levels refer to herd size)	Average herd size as number of animals over the time period 2016 to 2018	210.90 (180.48-242.32)	129.83(124.60-135.06)	0.968	0.892-1.043	0.41

Table 3: Results of final multivariable case control study containing calculated effects for the two way interactions included in the model.

Variable interactions		Odds Ratio	95% CI
Mixed grazing of cattle and sheep	**Do cattle drink from natural sources of water**		
yes	no	1	-
yes	yes	0.651	0.286-1.481
Divisional Veterinary Office of the bTB breakdown	**Any woodland on the farm or within 1.6km of the farm**		
Armagh, Newry, Newtownards (South East)	no	1	-
	yes	0.520	0.201-1.207
Dungannon, Enniskillen, Strabane, Omagh (West)	no	1	-
	yes	0.492	0.205-1.252
Ballymena, Coleraine, Mallusk (North East)	no	1	-
	yes	0.453	0.161-1.271
Divisional Veterinary Office of the bTB breakdown **(Figure 2)**	**Herd size transformed (increase in value by one)**		
Armagh, Newry, Newtownards (South East)	no	1	-
	yes	0.968	0.892-1.043
Dungannon, Enniskillen, Strabane, Omagh (West)	no	1	-
	yes	1.005	0.907-1.132
Ballymena, Coleraine, Mallusk (North East)	no	1	-
	yes	1.069	0.899-1.124

Table 3 continued

Variable interactions		Odds Ratio	95% CI
bTB breakdown risk picked as most likely source by VO **(Figure 3)**	**Herd size transformed (increase in value by one)**		
Source of infection not established (includes Other and Deer source)	no	1	-
	yes	0.968	0.892-1.043
Cattle to cattle contiguous herd spread	no	1	-
	yes	1.046	0.993-1.173
Purchase of infected animal(s)	no	1	-
	yes	1.119	0.993-1.261
Carryover of previous infection	no	1	-
	yes	1.002	0.865-1.161
Badgers	no	1	-
	yes	1.071	0.954-1.203
Purchase of store cattle at a market within the previous five years. **(Figure 4)**	**Herd size transformed (increase in value by one)**		
yes	no	1	-
yes	yes	1.050	0.950-1.160

Figure 1: Northern Ireland DVOs aggregated into three groups, southeast, northeast and west.

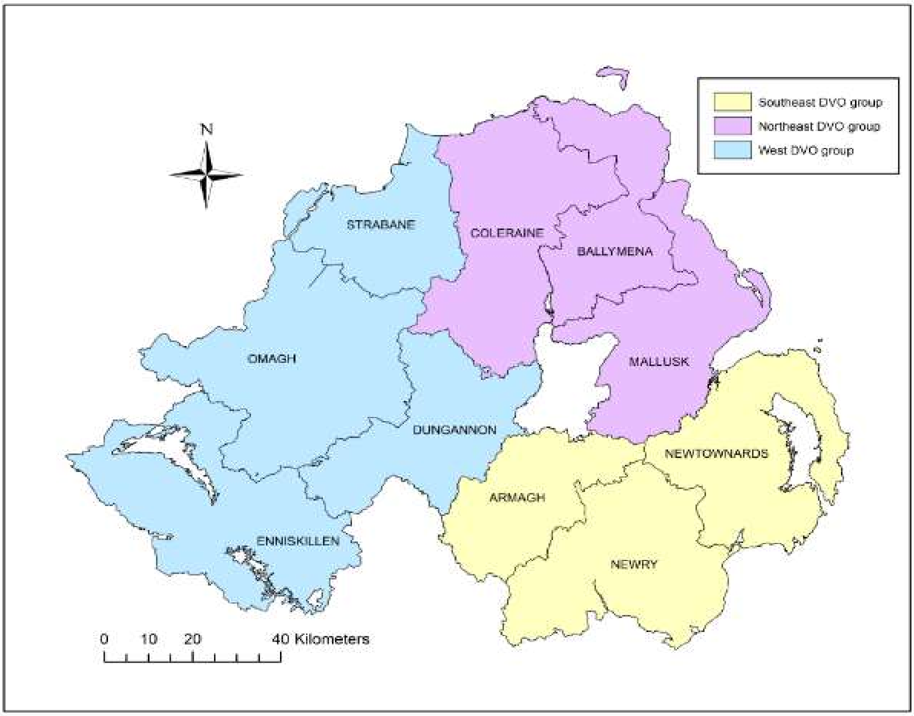


Figure 2: Duration case odds ratio for DVO of herd given effect of increasing herd size (variable herd size graphed in untransformed state).

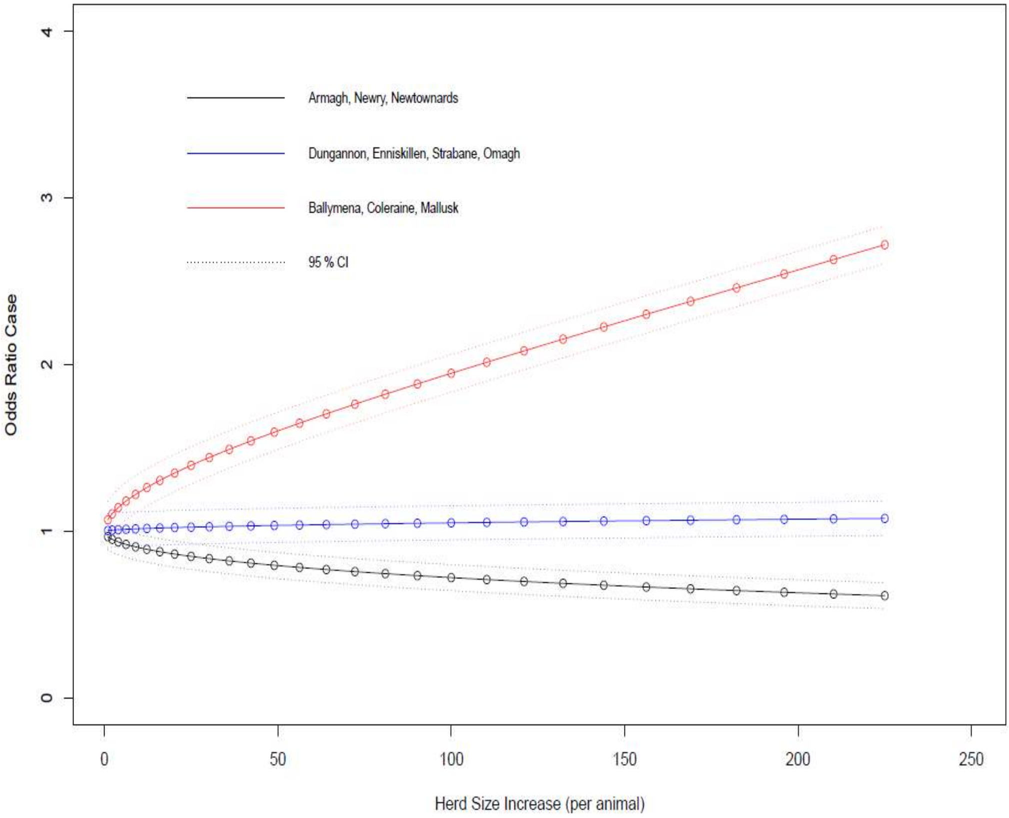


Figure 3: Duration case odds ratio for bTB breakdown by most likely VO source given effect of increasing herd size (variable herd size graphed in untransformed state).

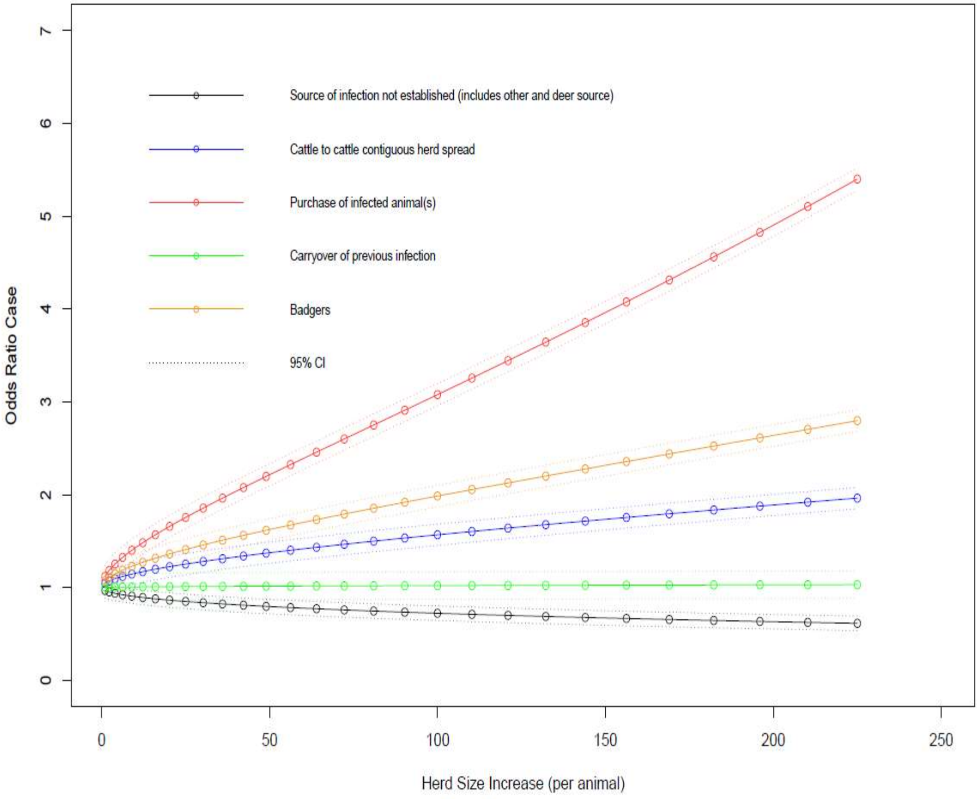
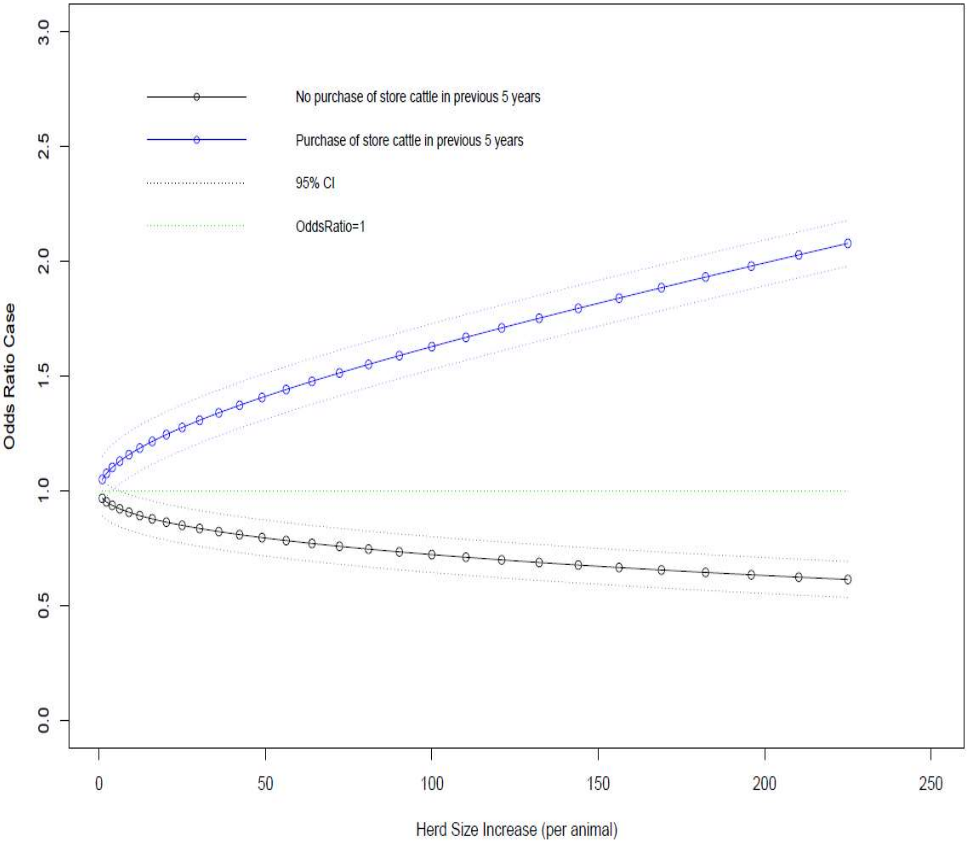


Figure 4: Duration case odds ratio for purchase of store cattle in the previous five years given the effect of increasing herd size (variable herd size graphed in untransformed state).



Chapter 7

Northern Ireland Farm Level Management Factors for Recurrent Bovine Tuberculosis Herd Breakdowns

Submitted for review to Epidemiology and Infection

Original Paper

Farm Level Management Factors for Recurrent Bovine Tuberculosis Herd Breakdowns

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Running head: Bovine tuberculosis surveillance

SUMMARY

This study investigated farm management factors associated to recurrent bTB (bovine Tuberculosis) breakdowns disclosed in the period 23/05/2016 to 21/05/2018 and is a follow up to our 2020 paper which looked at long duration bTB breakdowns. A farm level investigation (n=2935) was completed when one or more SICCT (Single Intradermal Comparative Cervical Test) reactors or when one or more confirmed (positive histological and/or bacteriological result) LRS (lesion at routine slaughter) were disclosed. A case control study design was used to construct an explanatory set of management factors associated to recurrent bTB herd breakdowns; defined as any bTB breakdown of less than a year in duration followed by at least two further bTB breakdowns within the two following years. In Northern Ireland a Department of Agriculture Environment and Rural Affairs (DAERA) Vet investigates bTB breakdowns using standardised guidelines to allocate a disease source. In this study source was strongly linked to carryover of infection, demonstrating that the diagnostic tests had failed to clear herd infection during the restricted period. The study also investigated the role of herd type (dairy herds 43% of cases) and herd size in recurrent breakdown situations.

Key words: Epidemiology, *Mycobacterium bovis,* bovine tuberculosis, Case-Control Study, Chronic breakdowns, Cattle.

INTRODUCTION

Bovine tuberculosis (bTB) caused by *Mycobacterium bovis is* a zoonotic disease primarily affecting animals. Although cattle are the main hosts, the disease has been reported in many other farmed and wild animals [1]. A key element of the bTB eradication strategy for Northern Ireland [2] published in 2016 was the recognition that herds chronically infected with bTB ('chronic herds') should be investigated as a distinct entity for action and a package of measures developed so as to minimise their impact on bTB eradication. This present work which focuses on herds recurrently breaking down with bTB complements previous Northern Ireland work on the subject [3, 4] and aims to provide an accumulating evidence base for the development of effective policies. Addition of this present work to the other studies [3, 4] on the subject of chronic bTB will bring alignment between Northern Ireland and study of such management factors already completed in Great Britain and the Republic of Ireland [5-8].

Doyle et al. (2016) used data from a national database APHIS (Animal and Public Health Information System) to inform definitions for prolonged and recurrent bTB breakdowns in chronic herds [3, 9]. The follow up study on management factors for prolonged bTB breakdowns found that purchase of infected animal(s) had the strongest association as the most likely source of infection for long duration bTB herd breakdowns followed by badgers and then cattle-to-cattle contiguous herd spread [4]. It also demonstrated that two subpopulations of prolonged bTB breakdowns exist in Northern Ireland, the first being beef fattening herds with main source continuous purchase of infected animals and a second group of primary production herds (dairy, beef cows and mixed) with risk from multiple sources [4]. This present work focuses on farm level management factors for chronic bTB herds that had recurrent bTB herd breakdowns. In this context chronic recurrent infection is the situation where a herd with confirmed bTB, defined as Officially Tuberculosis Withdrawn (OTW), has this status removed post completion of statutory testing (derestriction) and replaced by Officially Tuberculosis Free (OTF) status (allowing it to trade with other herds); with this OTF/OTW/OTF cycle repeating many times in some herds before infection is cleared. When a herd breakdown recurs as a result of disease disclosure at a post outbreak herd test or as a result of an LRS (Lesion at routine slaughter) before the post outbreak herd test it is likely this occurred due to presence of infected but undetected animals at the time of derestriction [9]

Breakdown recurrence in Northern Ireland at the six monthly and yearly post outbreak herd tests measured as a percentage of the tests carried out in the previous year was 11% and 9%, respectively, which mimics that for the herd tests carried out at derestriction (allowing a herd to regain its OTF status), at 11% (DAERA unpublished data); its percentage only being surpassed by herd tests carried out at the beginning and during bTB herd breakdown events. Disclosure of bTB infection at the six-month post outbreak herd tests are demoralising for farmers and vets, which are interpreted as a previously wasted effort and are viewed as simply returning the farm business to the beginning of a protracted disease control process. This also crucially erodes confidence in the integrity of the whole bTB eradication programme. Irish work defined bTB recurrence as further bTB restrictions in the same herd within a specific time-frame, resulting from either residual undetected infection in cattle, residual infection due to contamination of fomites (such as slurry), contiguous inter-herd spread or reinfection from local wildlife, however of the sources listed residual infection is thought to lead to recurrence more quickly [9]. Other studies on bTB recurrence in herds generally investigated some or all of these sources of infection [9], the imperfect sensitivity of the tuberculin test [10] or bTB herd breakdown risk factors such as number of bTB reactors, herd size, retention of inconclusive bTB reactors and the herd bTB history [8, 11-16]. The percentage of herds retaining bTB infected animals after herd derestriction have also been modelled (up to 21% in the HRA (High Risk Area) in England) [17]. Our study is unique in that it looked for associations in recurrent bTB breakdowns to farm management factors for Northern Ireland herds, comparing situations where bTB breakdowns recur quickly to those which do not recur or recur at a slower rate, thus adding to the body of knowledge on the subject.

Study Objective

The objective of this study was to use a case control study design to identify farm level management factors associated with recurrent bTB herd breakdowns, using data collected during on-farm epidemiological investigations.

MATERIALS AND METHODS

Study design and data collection

A case control study was conducted on a study population consisting of all bTB herd breakdown investigations during the period 23/05/2016 to 21/05/2018. The methodology and model framework applied to this study were as previously described [4, 18] but are summarised here for clarity. Data collection involved completion of an on-farm investigation form when one or more SICCT (Single Intradermal Comparative Cervical Test) reactors or one or more confirmed LRS were disclosed in any Northern Ireland cattle herd. Confirmation of bTB in an LRS was defined as a positive histological and/or bacteriological culture result following laboratory examination. Investigations were carried out by trained Animal Health and Welfare Inspectors (AHWI) who visited each of the bTB breakdown farms. At each farm, an on-site questionnaire was completed (supplementary table S2) through face-to-face interview of the farmer, including identification of all herds contiguous to the bTB herd breakdown. Based on the completed questionnaire and local knowledge of the area, the DAERA Vet responsible for the bTB herd breakdown, where possible, determined the most likely source of infection for the breakdown. Questionnaire information along with data extracted from APHIS (herd size and location) were collated into Microsoft Access^{TM} (Microsoft Corporation, Redmond, WA, USA). For our study, the ten Divisional Veterinary Offices (DVOs) in Northern Ireland were aggregated into three groups according to their geographic location; south east group (Armagh, Newry, Newtownards), west group (Dungannon, Enniskillen, Strabane, Omagh) and north east group (Ballymena, Coleraine, Mallusk) (Figure 1).

Cases were bTB herd breakdowns of less than a year in duration followed by at least two further bTB herd breakdowns within the following two years [3] (three breakdowns within three years). A disease investigation carried out on a bTB herd breakdown in the period 23/05/2016 to 21/05/2018 and which was preceded by at least two bTB herd breakdowns in previous two years was defined as a case [3]. These cases defined in this study, had an associated duration of <365 days to ensure mutual exclusivity to the cases used in the previous prolonged duration study [4]). Controls were bTB herd breakdowns of less than a year in duration initiating during the study period (23/05/2016 to 21/05/2018) and which were linked to a maximum of one breakdown within the previous two years.

Data analysis

Microsoft Access^{TM} (Microsoft Corporation, Redmond, WA, USA) and R Version 3.4.0[5] were used for data manipulations and R Version 3.4.0[1] and Stata/SE 15[6] were used for data analysis. The model framework used was binary logistic regression using purposeful selection of covariates [18] with the case definition forming the response variable. A total of 78 explanatory variables were derived from the on-farm questionnaire (see supplementary table S2) along with their associated factor levels. Initially all variables were tabulated using the duration case definition against each variable's factor levels. As variables were added or removed from the model, the AIC (Akaike Information Criterion) difference was calculated between the old and new proposed model, in order to determine if the proposal reduced AIC by a value greater than two [18]. Where the models were subsets of each other, the LRT (Likelihood Ratio test) was also calculated in order to determine if addition or removal of variables was significant at the $P \leq 0.05$ level.

Initial analysis was by univariable logistic regression. Any variables containing low numbers (<10) of cases at any factor level, which could not be logically merged with another factor level were removed after univariable analysis. Remaining variables with $P \leq 0.25$ were then analysed using a multivariable logistic regression model. The resultant model was further refined to produce a reduced multivariable logistic regression model, which utilised variables with $P \leq 0.05$ from the first multivariable model. Following the fit of the reduced multivariable model, its estimated coefficients were compared to those in the initial multivariable model to determine if there was a magnitude change of > 20%. This magnitude change known as $\Delta\hat{\beta}\%$ (Delta-Beta-Hat %) indicated that one or more of the excluded variables were important in the sense of providing a needed adjustment effect of the variables that remained in the model [18]. Variables which formed the first multivariable model but were not included in the initial reduced multivariable model were added back individually; being retained if they contributed to the overall model and reduced $\Delta\hat{\beta}\%$ to below 20%.

Further to this, variables with $P > 0.25$ in the initial univariable analysis were also individually added back in, to determine if they contributed to the multivariable model thus producing the preliminary main effects model. The only continuous variable included in the preliminary main effects model was herd size. Fractional polynomial analysis [19] was applied to herd size in order to determine if it required scale transformation so as to satisfy the assumption of linearity in the logit outcome. Completion of this stage produced the main effects model. Using the variables present in the main effects model, all combinations of two way interactions were statistically assessed using the LRT ($P \leq 0.05$); however, only those with probable biological significance were accepted as potential candidates for the model. Interaction terms accepted into the final model had an odds ratio calculated as a linear combination with their associated main effects ($\beta_0 + \beta_1 + \beta_2 + \beta_1.\beta_2$). The finalized model was then subjected to the Hosmer-Lemeshow goodness-of-fit-test (decile sub-grouped) to determine its fit to the data. Table 1 details how the methodology was applied in this study to achieve the final multivariable model.

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[5] R Core Team (2017). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL https://www.R-project.org/.

[6] StataCorp. 2017. Stata Statistical Software: Release 15. College Station, TX: StataCorp LP.

RESULTS

A total of 2935 bTB herd breakdown investigations (192 cases and 2743 controls) were completed during the study period (summary details in supplementary table S1). Controls were confirmed bTB breakdowns where there was no breakdown in the previous two years (n=1924) or one breakdown (n=819). The results from each stage of the model building process from univariable analysis through to the final multivariable model are detailed in Table 1. As a result of carrying out fractional polynomial analysis [19] on herd size it was transformed to herd size to power 0.5 and was then referred to as 'herd size transformed' in the subsequent analysis.

The results from the final model (Table 2) demonstrated that type of farming enterprise was a significant variable in the final multivariable model, however the factor level odds ratios were not statistically different from each other at the $P \leq 0.05$ level. In terms of farm enterprise type dairy enterprises (reference category) formed 43% of cases. The potential for badgers to access silage clamps was found to be statistically significant (P=0.013; OR=1.752; 95%: 1.137-20775) while the application of slurry/manure to farmland mostly in the spring time as opposed to continuously over the grazing season was protective (P=0.27; OR=0.672; 95%CI: 0.475-0.961). The OR of a case in relation to geographical/other factors (Table 2) were as follows; where particular parcels of ground were associated with TB in cattle was 1.676 (95%CI: 1.152-2.411); where there was partial upgrading of boundary fences in the last three years was 1.804 (95% CI: 1.154-2.883); where there was full upgrade of boundary fences in the last three years was 1.247 (95% CI: 0.801-1.981); where there was presence of badger setts locally but not on the investigation farm was 1.368 (95%CI: 0.979-1.905); where there was any woodland on the farm or within 1.6km from the farm was 0.648(95%CI 0.470-0.895); where a farm is located in DVO west group was 1.328 (95%CI: 0.881-2.037) and with DVO north east group was 1.675 (95%CI: 1.066-2.662); with silage fields after grazed by cattle was 1.918 (95%CI 1.117-3.528);

With respect to source of infection for cases (Table 2), the OR for cattle to cattle contiguous herd spread was 0.890 (95%CI: 0.554-1.424), for purchase of infected animal(s) source was 1.349 (95%CI: 0.797-2.266), for carryover of infection source was 2.565 (95%CI: 1.561-4.198) and for badger infection source was 0.914 (95%CI: 0.560-1.482). The presence of a badger sett was recorded on 30% (95% CI: 28.4-31.7) of investigations and of these 3% (95% CI: 2.3-3.6%) reported fencing the sett/latrine off. Of cases which were determined to have a breakdown source as carryover of infection 57.5% (95%CI: 42.2-72.8%) were dairy enterprises, 25% (95%CI: 11.6-38.4%) were beef cow enterprises, 15% (95%CI: 3.9-26.1%) were beef fattening enterprises and 2.5% (95% CI: 0.0-7.3%) were other mixed enterprises.

Along with the main effects, addition of two interaction terms, 'finding a dead badger on your farm in the past three years' x 'herd size transformed' and 'total parcels of land grazed' x 'herd size transformed' improved the model. The results of the linear combination of these interaction terms and their associated main effects ($\beta_0 + \beta_1 + \beta_2 + \beta_1.\beta_2$) are shown in Table 3 and Figures 2 and 3. Results from Table 3 show that the odds ratio of a case where a dead badger was observed on the farm in the previous three years and there was an increase in herd size transformed by one was 0.997 (95%CI: 0.880-1.070). Figure 2 shows the effect on the recurrence case definition of increasing herd size (untransformed) in situations where a dead badger was observed on the farm in the previous three years. The results of figure 2 show that when herd size is taken into account, the finding of a dead badger does not lead to an odds ratio of a recurrent case which differs significantly from one.

Figure 2 also shows that there is a strong association between increased odds ratio of a recurrent breakdown and increasing herd size. Within the recurrence case group, the mean herd size was 202 (95%CI: 182-223) and median herd size 158 (IQR: 93-266) animals. In this study control herds were also bTB breakdowns with mean herd size 130 (95%CI: 125-136) animals and median 80 (IQR: 38-172). In the Northern Ireland cattle industry as a whole average herd size in 2019 was 76.0 animals (2019 TB testing data) [20].

The interaction variable 'total parcels of land grazed' x 'herd size transformed' had a significant interaction on addition to the main effects model (LRT: P=0.01; Table 3). The odds ratio of a case where one parcel of land is used for grazing cattle and there was an increase in herd size transformed by one had was 1.108 (95%CI: 1.035-1.182), where two parcels of land was used and herd size transformed increased by one was 1.143 (95%CI: 1.023-1.277), where three parcels of land were used and herd size transformed increased by one was 1.108 (95%CI: 0.997-1.232), and with four or more parcels of land and herd size transformed increase by one was 1.017 (95%CI: 0.919-1.126). Figure 3 shows the effect on the recurrence case definition of increasing herd size (untransformed) at the different levels of the number of land parcels used for grazing cattle variable. In terms of recurrent cases where one parcel of land was used to graze cattle this group was made up of 21.2% (95%CI: 7.3-35.1%) dairy enterprises, where two parcels of land was used to graze cattle 57.7% (95%CI: 43.3-72.2%) were dairy enterprises, where three parcels of land was used to graze cattle 49.1% (95%CI: 36.1-62.1%) were dairy enterprises where four or more parcels of land were used to graze cattle 38.6% (95%CI: 25.9-51.2%) were dairy enterprises.

DISCUSSION

This work delivers an insight into recurrent bTB breakdowns in Northern Ireland, complementing a previous study on chronic bTB which looked at prolonged breakdowns [4]. It looked specifically at farm management factors, an area which has not been previously investigated using Northern Ireland data and, by using cases disclosing three or more bTB breakdowns within three years, provides an unique comparison of the more extreme end of the spectrum for recurrence.

Data used in this study were derived from two sources; the farmer experiencing the bTB breakdown and a trained DAERA Vet who managed the breakdown and allocated a source using standardised guidance. With our previous study [4] variables relating to purchase of cattle were identified by farmer and DAERA Vet responses as significant contributors to prolonged breakdowns. This contrasts with this parallel study on recurrent breakdowns where variables relating cattle purchases were not significant management factors; a finding which is similar to that observed in GB [8]. An Irish study [9] hypothesised that persistent infection in a herd due to residual infection in cattle would be expected, on average to lead to recurrence more quickly than re-infection from other sources. Our results indicate that carryover of infection from a previous breakdown was the only level of this factor found statistically significant, hence concurring with the Irish study conclusions [9].

This result is not surprising given the imperfect sensitivity of the SICTT [10] and changes have been applied to the Northern Ireland bTB eradication programme aimed at increasing its effectiveness. Recent changes have included enhanced removal of inconclusive SICTT reactors [21-23], enhanced application of more stringent test cut off values (severe interpretation) [21, 22], increased number of breakdowns subjected to a minimum 120 day restricted period and minimum two SICTT herd tests [21, 22], application of parallel IFNG (Interferon Gamma) testing to enhance test sensitivity [20, 24] and also broader initiatives such as introduction in 2016 of a PVP (Private Veterinary Practitioner) contract, which aimed to improve overall quality of bTB testing [20].

In contrast to the DAERA Vets who pointed to an unambiguous source of infection for recurrent breakdowns, farmers' responses returned associations to a much wider spectrum of management factors. Type of farming enterprise was a significant variable throughout the model building process. Dairy farming enterprise was taken as the baseline category and relative to it, none of the other levels showed a significant difference ($P<0.05$). In terms of recurrent cases, 43.2% were dairy farm enterprises which is a large representation given that approximately 10% of Northern Ireland farming enterprises are defined as dairy [25], however results did not show dairy farm enterprises at a significantly greater risk than other herd types. Previous work in GB and ROI [8, 14] studied the effects of herd type in recurrent breakdowns, the former study including dairy herds as a risk factor, while the ROI study did not include it in the final multivariable model. However both studies [8, 14] acknowledged a relationship between herd type and herd size as influencing which of these variables appear in final models. Notably our study included both farm enterprise and herd size in the final model. As in GB and ROI, in Northern Ireland the relationship between herd type and farm enterprise shows that dairy farms are generally larger than beef breeding farms (average herd size 95 vs 15 cows, respectively [25]). However, compared to the general population, our study only involved bTB breakdowns which are more likely to occur in larger herds (average control herd size was 130.4 (95%CI: 124.92-135.86). Herd size arguably provides a statement summarising the increased bTB risk exposures experienced relatively by large herds. These risks include the need to graze larger areas, risking increased contact with infectious wildlife, more purchase and movement of cattle, larger numbers of contiguous herds, more intensive management with higher production stress and, if infected, more difficulty in clearing infection due to the poor sensitivity of skin tests allowing infected animals with false negative results remaining in the herd [26, 27].

In addition to the direct risk from herd size there has been a move towards intensification in Britain and Ireland with a trend towards larger farms and decline in absolute numbers [26], probably making the problem more intractable (in Northern Ireland, there has been almost a 1% annual decline in the number of cattle herds over the last 15 years while cattle number have remained relatively constant [28]). With our study, herd size also had an interaction effect with both finding a dead badger on the farm in the previous three years and number of land parcels used for grazing cattle. In the case of finding a dead badger on the farm, this variable did not have a significant association to the case definition when interpreted at increasing levels of herd size (figure 2). A previous Northern Ireland study reported an association between the finding of badger carcases on the farm and bTB breakdown [29]. Indeed even with number of parcels of land used for grazing cattle what appeared to be an increasing strength of association linked to more parcels became less clear when interpreted as an interaction with herd size (figure 3). When interpreted as an interaction with herd size, only the category using two parcels of land had a statistically significant odds ratio greater than the baseline (one parcel); a group made up of 57.7% dairy enterprises. This result may reflect a situation in large herds where they are managed as several smaller units making it easier to clear infection from a particular group of cattle, which in terms of diagnostic test performance functions as a ‘small’ herd. Further investigations are required to disentangle these findings especially its significance in relation to type of farm (dairy vs non-dairy) [30].

As already noted [9] the recurrence of bTB breakdowns could originate from sources other than carryover of infection, such as contamination of fomites such as slurry, contiguous spread or reinfection from local wildlife. A significant reduction in the odds ratio of a recurrent bTB breakdown was recorded when slurry was mostly applied in the spring time, rather than continuously over the grazing season. This factor may be indicative of farming intensity where land availability is limited to enable application of the majority of the slurry early in the grazing season. The direct risk of infection to cattle from the slurry application process [5] is probably increased if continued over the grazing season when direct exposure to cattle is more likely.

In terms of contiguous spread, recent upgrading of boundary fences was significant. However, the interpretation is unclear as it suggested that partial upgrade of boundary fences leads to an increase in recurrence while full upgrade was not a statistically significant factor level. One explanation might be that farmers suffering recurrent breakdowns were upgrading boundary fences as a reactive biosecurity measure, which was ineffective as, in many cases, the bTB source was carry over of infection in their own herd. Another significant variable which could be related to both boundary fencing and wildlife in the locality was a farmer highlighting specific land parcels they associated with bTB in their cattle. This variable however has limitations in terms of its interpretation as such land parcels could be adjacent to herds at high risk of bTB breakdowns or favourable to wildlife/badger habitats, which if infected could act as a source for recurrent bTB herd breakdowns.

Two other significant variables related to badgers were access to silage clamps and the presence of badger setts in the locality but not on the investigation farm. However, similar to the previous study [4], results indicated that 30% of farms investigated claimed to have a badger sett present but only 3% of farms actively fence off setts to prevent cattle accessing them. Another study in NI demonstrated that cattle visit badger setts/location three times more frequently than badgers visit cattle locations [31] affirming that this lack of such a biosecurity barrier may be an important finding in preventing this potential indirect infection pathway.

Grazing cattle on fields after silage removal was also significantly related to the cases. This variable could be a proxy for farms with more intensive production, also allowing more exposure to contiguous herds [32]. However, it is a practice which forces cattle to graze around a field perimeter possibly exposing them to badger setts and latrines which are more likely to be located in hedgerows in a Northern Ireland setting [33-36] and moreover, very unlikely to be fenced off.

As with our previous study [4], this study also showed that herds located in the DVO north east group (figure 1) have the strongest statistical association to the recurrent bTB herd breakdowns. Also included and showing a reduced odds ratio for its association to cases was the presence of woodland on the farm or within 1.6km of the farm. Variables relating to woodland in the farm vicinity has been included in studies previously [5, 37] but were not significant. It is a possible that in Northern Ireland woodland could act as a proxy for areas adjacent to coniferous plantations which are predominantly in areas of poorer land quality and smaller cattle herds.

In this study the final multivariable model contained 13 variables and given the use of a statistical cut off level of $P<0.05$ for variable selection, there is a possibility that at least one spurious variable has been included. It is also possible with a study design where DAERA Vet’s select a breakdown source of infection there is potential for a degree of subjectivity. However, the DAERA Vet with their local knowledge, training and standardised guidance is best placed to make these assessments.

CONCLUSIONS

One of the most important tasks a DAERA Vets performs is investigating bTB herd breakdowns where, after careful consideration using standardised guidelines, they allocate a disease source. In the situation where there was a high frequency bTB herd breakdown pattern (three or more individual bTB breakdowns within a three year period), this study showed that DAERA Vets strongly associated the infection source to be carry over of infection, indicating that the diagnostic tests have failed to clear herd infection in the time allocated to the restricted period for these cases. In terms of management factors associated to recurrent bTB herd breakdowns, the size of the herd and location in north-eastern Northern Ireland were significant, along with indicators of increased production intensity such as extended slurry spreading on grassland and after-grazing of silage fields. Herd type was a very strong contender variable in the final model, with dairy herds making up 43% of cases; however factor level results did not show them to be statistically more at risk than other herd types. Even though badgers were not highlighted as a significant source of infection in this study, it cannot be ignored that so little effort is applied to create a biosecurity barrier between their setts/latrines and grazing cattle.

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CONFLICT OF INTEREST

None.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study is not publically available data.

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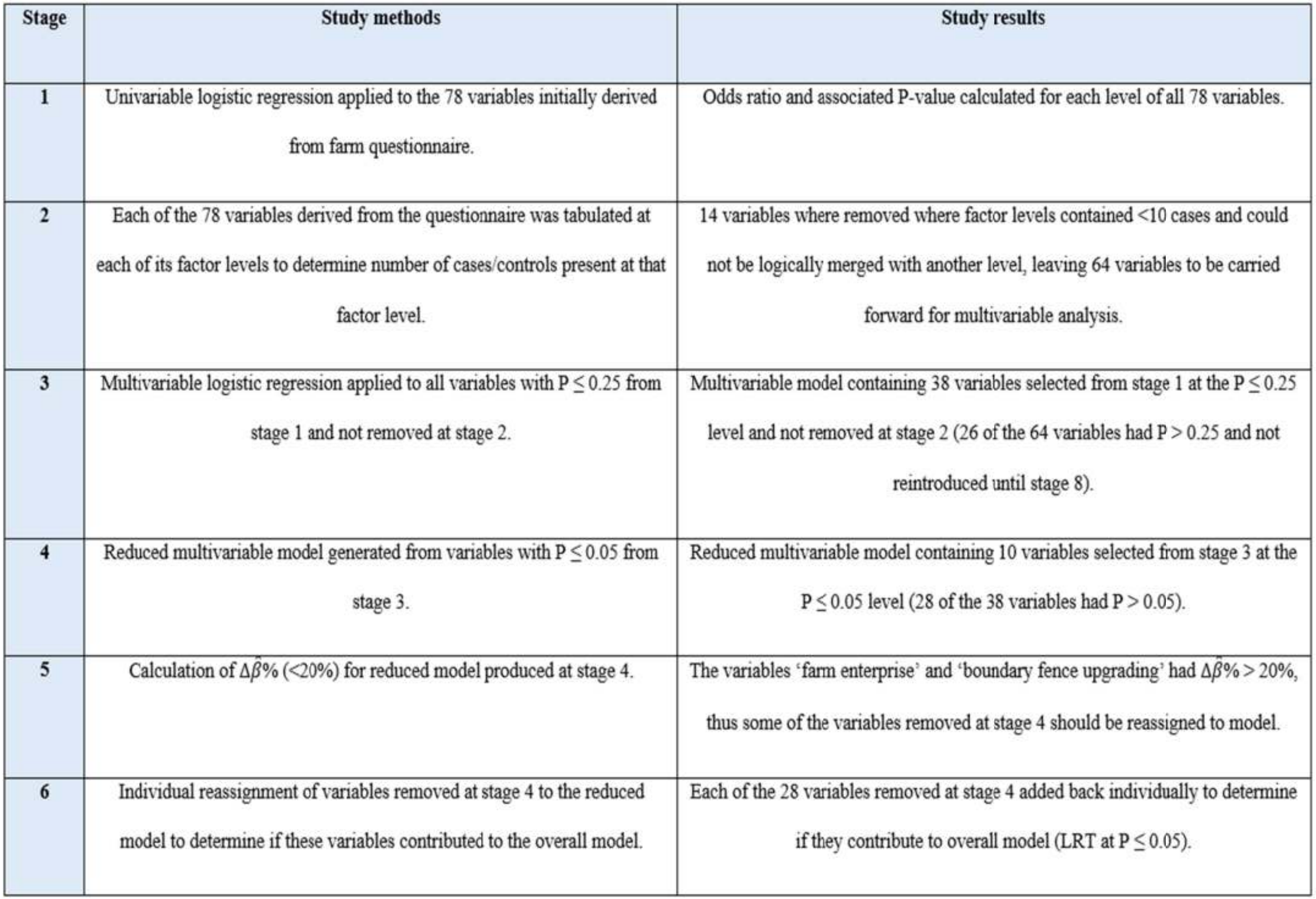
Table 1: Table showing methods applied and results observed at each stage of the study model building process.



Stage	Study methods	Study results
1	Univariable logistic regression applied to the 78 variables initially derived from farm questionnaire.	Odds ratio and associated P-value calculated for each level of all 78 variables.
2	Each of the 78 variables derived from the questionnaire was tabulated at each of its factor levels to determine number of cases/controls present at that factor level.	14 variables where removed where factor levels contained <10 cases and could not be logically merged with another level, leaving 64 variables to be carried forward for multivariable analysis.
3	Multivariable logistic regression applied to all variables with $P \leq 0.25$ from stage 1 and not removed at stage 2.	Multivariable model containing 38 variables selected from stage 1 at the $P \leq 0.25$ level and not removed at stage 2 (26 of the 64 variables had $P > 0.25$ and not reintroduced until stage 8).
4	Reduced multivariable model generated from variables with $P \leq 0.05$ from stage 3.	Reduced multivariable model containing 10 variables selected from stage 3 at the $P \leq 0.05$ level (28 of the 38 variables had $P > 0.05$).
5	Calculation of $\Delta\hat{\beta}\%$ (<20%) for reduced model produced at stage 4.	The variables 'farm enterprise' and 'boundary fence upgrading' had $\Delta\hat{\beta}\% > 20\%$, thus some of the variables removed at stage 4 should be reassigned to model.
6	Individual reassignment of variables removed at stage 4 to the reduced model to determine if these variables contributed to the overall model.	Each of the 28 variables removed at stage 4 added back individually to determine if they contribute to overall model (LRT at $P \leq 0.05$).

Table 1 continued

Stage	Study methods	Study results
7	Reduced multivariable model refined by variable addition/removal to obtain $\Delta\bar{\beta}\%$ <20% for all variables included in the multivariable model.	Refining of reduced multivariable model from stage 4 results in addition of three new variables: 1. Silage fields after grazed with cattle. 2. Herd size. 3. Total parcels of land grazed. All $\Delta\bar{\beta}\%$ for variables in the multivariable model at this stage of model building were <20%.
8	Individual reassignment of variables removed at stage 3 to the model produced at stage 7 to determine if these variables contributed to the overall model. Output from this stage formed the preliminary main effects model.	Each of the 26 variables removed at stage 3 were added back individually to determine if they contribute to the overall model (LRT at $P \leq 0.05$). None of these variables were deemed to meet the criteria for addition to the model.
9	Fractional polynomial analysis to assess linearity of any continuous variables to the outcome.	Analysis results in the variable transformation of 'herd size' to a power of 0.5. 'Herd size' was added in its transformed state to the preliminary model to form the main effects model and was referred to as 'herd size transformed'.
10	All combinations of two way interactions (LRT at $P \leq 0.05$ and judged as biologically significant) from main effects model were added to main effects model. Interactions retained or removed based on p-value ($P \leq 0.05$) within the model and as to whether they lead to model improvement (AIC and LRT cut offs already described). These interaction variable pairs were then	Two interaction terms were added to the main effects model of 13 variables to form the final model. Odds ratio for these interactions in linear combination with their main effects were calculated and added to Table 3.

Table 1 continued

Stage	Study methods	Study results
	evaluated as a linear combination ($\beta_0 + \beta_1 + \beta_2 + \beta_1.\beta_2$) with the main effects to determine their overall odds ratio and associated confidence interval (Table 3).	1. Observing a dead badger on the farm in the last three years x Herd size transformed (LRT: P=0.006) 2. Total parcels of land grazed x Herd size transformed (LRT: P=0.01)
11	Application of the goodness of fit test (Hosmer and Lemeshow goodness of fit test) and variable correlation analysis to the final model.	Hosmer and Lemeshow goodness of fit test result: P= 0.5987. Provides evidence at $P \leq 0.05$ level of adequate goodness of fit.

Table 2: Results of final multivariable case control study containing categorical and continuous variables (note interaction terms are included in table 3).

Study Variable Categorical	Exposure Level	Case: (n=192)		Control: (n=2743)		Odds Ratio	95% CI	P-Value
	(NA=Not Applicable)	n	%	n	%			
Type of farming enterprise	Dairying	83	10.85	682	89.15	-	-	-
	Beef cows	36	3.36	1035	96.64	0.638	0.390-1.034	0.071
	Beef fattening	39	6.14	596	93.86	1.194	0.716-1.972	0.492
	Beef cows and fattening	16	5.23	290	94.77	0.644	0.345-1.143	0.148
	Mixed enterprise	18	11.39	140	88.61	1.127	0.608-2.002	0.693
Could badgers potentially access silage clamps?	No	29	2.94	959	97.06	-	-	-
	Yes	163	8.37	1784	91.63	1.752	1.137-2.775	0.013
Is slurry/manure applied mostly in spring time rather than continuously over grazing season?	No	54	7.64	653	92.36	-	-	-
	Yes	138	6.19	2090	93.81	0.672	0.475-0.961	0.027

Table 2 continued

Study Variable Categorical	Exposure Level	Case: (n=192)		Control: (n=2743)		Odds Ratio	95% CI	P-Value
	(NA=Not Applicable)	n	%	n	%			
Do you have particular parcels of land that you associate with TB in your cattle?	No	139	5.72	2293	94.28	-	-	-
	Yes	53	10.54	450	89.46	1.676	1.152-2.411	0.006
Any boundary fence with a neighbour upgraded in the past three years (where full upgrade is installation of a complete new fence)?	No	31	4.30	690	95.70	-	-	-
	Some upgrading	77	7.27	982	92.73	1.804	1.154-2.883	0.011
	Full upgrading	84	7.27	1071	92.73	1.247	0.801-1.981	0.338
Are you aware of any badger setts in the locality but not on your farm?	No	110	5.51	1887	94.49	-	-	-
	Yes	82	8.74	856	91.26	1.368	0.979-1.905	0.065
	No	162	5.86	2602	94.14	-	-	-

Table 2 continued

Study Variable Categorical	Exposure Level	Case: (n=192)		Control: (n=2743)		Odds Ratio	95% CI	P-Value
	(NA=Not Applicable)	n	%	n	%			
Have you seen a dead badger on your farm in the last 3 years?	Yes	30	17.54	141	82.46	9.477	2.989-29.863	0.000
Any woodland on the farm or within 1.6km of the farm?	No	95	7.61	1154	92.39	-	-	-
	Yes	97	5.75	1589	94.25	0.648	0.470-0.895	0.008
Divisional Veterinary Office of the bTB breakdown	Armagh, Newry, Newtownards (South East group	37	4.47	790	95.53	-	-	-
	Dungannon, Enniskillen, Strabane, Omagh (West group)	88	6.26	1317	93.74	1.328	0.881-2.037	0.183
	Ballymena, Coleraine, Mallusk (North East group)	67	9.53	636	90.47	1.675	1.066-2.662	0.027

Table 2 continued

Study Variable Categorical	Exposure Level	Case: (n=192)		Control: (n=2743)		Odds Ratio	95% CI	P-Value
	(NA=Not Applicable)	n	%	n	%			
Do you after graze your silage fields with cattle?	No or NA	15	3.94	366	96.06	-	-	-
	Yes	177	6.93	2377	93.07	1.918	1.117-3.528	0.026
bTB breakdown risk picked as most likely source by DAERA Vet	Source of infection not established (includes Other and Deer source)	44	4.88	857	95.12	-	-	-
	Cattle to cattle contiguous herd spread	39	5.86	627	94.14	0.890	0.554-1.424	0.629
	Purchase of infected animal(s)	33	6.35	487	93.65	1.349	0.797-2.266	0.260
	Carryover of previous infection	40	18.78	173	81.22	2.565	1.561-4.198	0.000
	Badgers	36	5.67	599	94.33	0.914	0.560-1.482	0.715
What is the total number of land parcels you use for grazing cattle?	One parcel of land used	33	4.09	774	95.91	-	-	-
	Two parcels of land used	45	6.12	690	93.88	0.806	0.253-2.543	0.714

Table2 continued

Study Variable Categorical	Exposure Level	Case: (n=192)		Control: (n=2743)		Odds Ratio	95% CI	P-Value
	(NA=Not Applicable)	n	%	n	%			
	Three parcels of land used	57	9.06	572	90.94	1.45	0.476-4.422	0.512
	Four or more parcels of land used	57	7.46	707	92.54	3.423	1.178-10.096	0.024
Study Variable Continuous	**Exposure level**	**Mean (95% CI)**				**Odds Ratio**	**95% CI**	**P-Value**
		Case		**Control**				
Herd size transformed	Average herd size as number of animals over the time period 2016 to 2018	202.47 (181.54-223.40) (Untransformed value)		130.39 (124.92-135.86) (Untransformed value)		1.108	1.035-1.182	0.002

Table 3: Results of final multivariable case control study containing calculated effects for the two way interactions included in the model.

Variable interactions		**Odds Ratio**	**95% CI**
Observing a dead badger on your farm in the last 3 years?	**Herd size transformed (increase in value by one)**		
yes	no	1	-
yes	yes	0.997	0.880-1.070
What is the total number of land parcels you use for grazing cattle?	**Herd size transformed (increase in value by one)**		
One parcel of land used	no	1	-
	yes	1.108	1.035-1.182
Two parcels of land used	no	1	-
	yes	1.143	1.023-1.277
Three parcels of land used	no	1	-
	yes	1.108	0.997-1.232
Four or more parcels of land used	no	1	-
	yes	1.017	0.919-1.126

Figure 1: Northern Ireland DVOs aggregated into three groups, southeast, northeast and west.

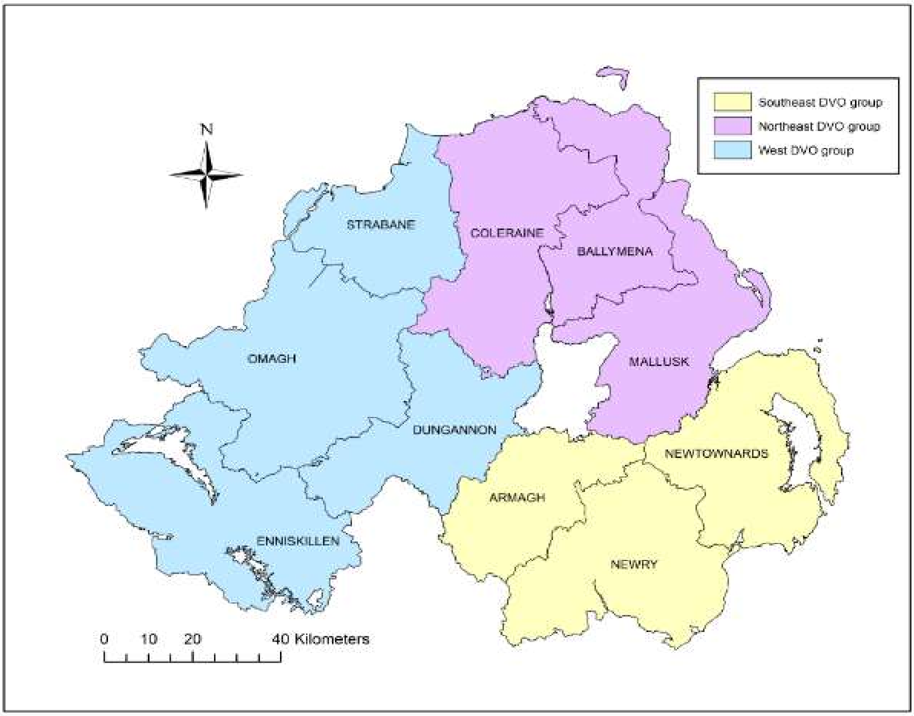


Figure 2: Recurrence case odds ratio for observing a dead badger on a farm given effect of increasing herd size (variable herd size graphed in untransformed state).

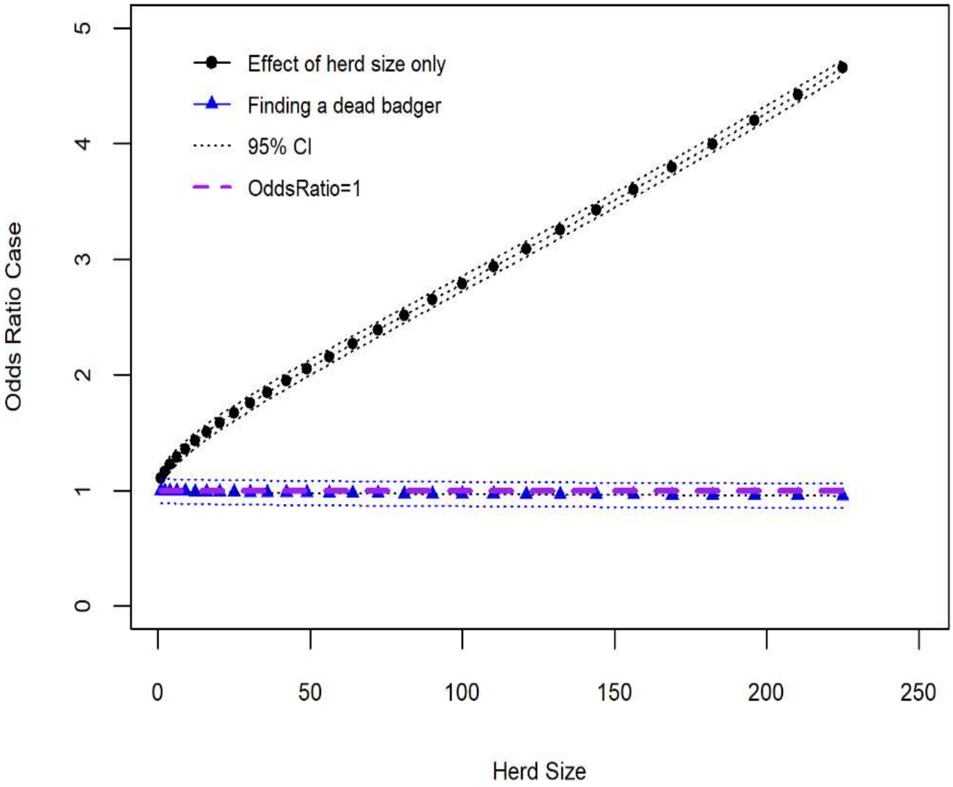
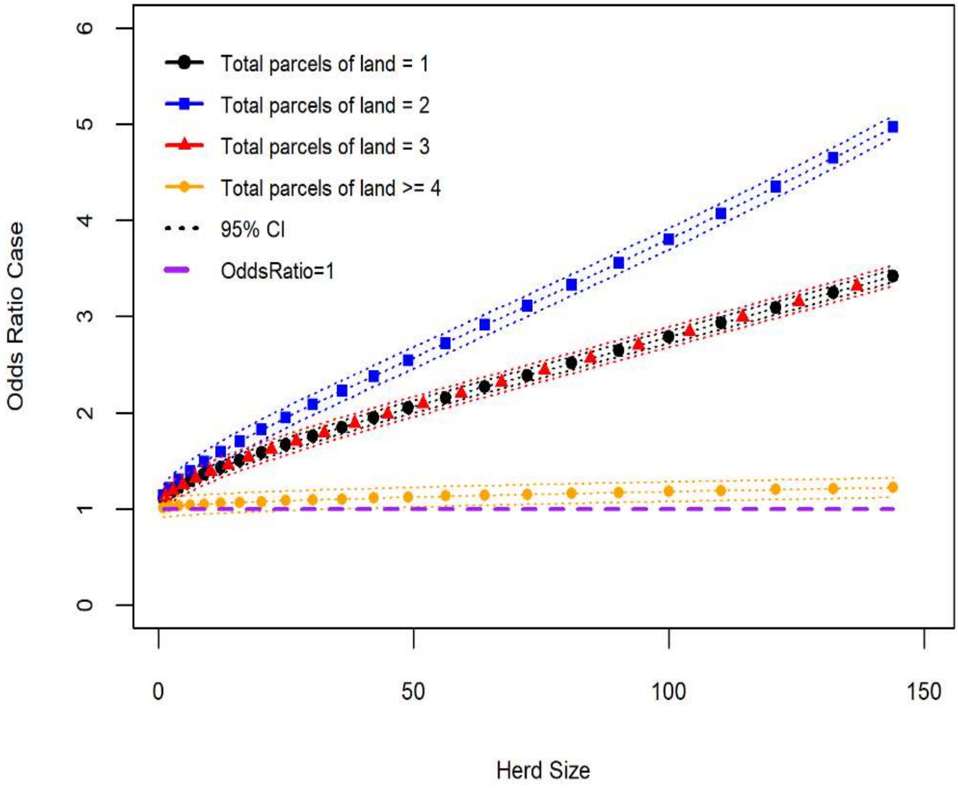


Figure 3: Recurrence case odds ratio for bTB breakdown by number of parcels of land used for grazing given effect of increasing herd size (variable herd size graphed in untransformed state).



Chapter 8

Research Overview and General Discussion

Research Overview

Chapter 2 of this dissertation explored recrudescence of bovine tuberculosis (bTB) in Northern Ireland (NI) herds by assessing risk factors associated with time from the six-month post-outbreak skin test until a further herd breakdown. This research was important as it investigated why after completing statutory testing requirements, disclosing infections at six monthly and yearly post breakdown herd tests (measured as a percentage of the tests carried out in the previous year) occurred frequently (DAERA, unpublished data). This work showed that breakdown severity (defined as the number of Single Intradermal Comparative Cervical Tuberculin (SICCT) test reactors at the disclosure test), local bTB prevalence, herd size and type were identified as significant factors, as was the purchase of cattle. This study has contributed to a better understanding of how herds which disclosed a bTB breakdown suffered a future breakdown. It was also used as part of the evidence base to change NI bTB policy in March 2018 so that an additional post-breakdown bTB herd test (CH2 – Check Herd Test 2) was carried out after any Officially Tuberculosis Withdrawn (OTW) herd breakdown. This policy change has the effect of detecting infected herds at an earlier stage, thus reducing the risk to uninfected herds.

Chapter 3 began formal investigations as part of the 'Chronic bTB Herds Project'. The purpose of this study was to use data in the national database APHIS (Animal and Public Health Information system) [1] to perform an analysis of potential associations to prolonged and recurrent bTB herd breakdown periods. This exploratory work also had the purpose of generating definitions based on NI data for prolonged and recurrent bTB herd breakdowns; which post peer review provided the basis for Chapters 6 and 7. The case definitions generated in Chapter 3 for a prolonged bTB herd breakdown was any confirmed bTB disclosure with duration greater than one year. The case definition for a recurrent bTB herd breakdown was any confirmed bTB disclosure with duration less than one year, followed by two or more bTB herd breakdowns within 2 years from the end of the initial TB herd breakdown.

Cattle movement is a commonly cited explanatory factor associated to bTB herd breakdowns and has been highlighted in both studies carried out for Chapters 2 and 3 of this dissertation. Given its contribution to bTB in NI and the fact that no local quantification of its effect had ever been carried out, Chapter 4 investigated this topic, helping to clarify its role and provide insight to it. One of the important aspects of this work was a design which controlled for the infectious process occurring within the disclosing herd, a feature which makes quantifying the source much more difficult. Results showed that 6.4% (95% CI: 5.9-6.8) of bTB herd breakdowns in NI were directly attributable to the movement of infected animals.

Chapter 5 was a collaborative study carried out between the countries of the British Isles known as the 'TB TRENDS project' and updated an earlier publication [2]. The author of this dissertation co-ordinated the NI part of this work and developed the new data structures required to report updated disease metrics, which were not achievable in the original publication. The important aspect of this work was the provision of standardised definitions and measures; allowing individual countries the capacity to directly compare their disease levels without the barrier of individualised disease measures applied in national statistics. This work describes trends in bTB from 2003 to 2015 for the countries of the UK (United Kingdom) and the ROI (Republic of Ireland).

Chapter 6 was a study grouped as part of the ‘chronic Tuberculosis herd project’ and used the definition from Chapter 3 to look at the farm management factors associated with prolonged bTB herd breakdowns (defined as bTB herd breakdowns of more than one year in duration). This study demonstrated for the first time that herds with prolonged bTB herd breakdowns were in fact two sub-populations based on their source of infection; beef fattening herds with the main infection source being continuous purchase of infected animals and a second group of primary production herds (dairy, beef cows and mixed) with risk from multiple sources. Another important observation from this study highlighted biosecurity issues where almost one third of bTB herd breakdown investigation reported that the associated farm contained a badger sett but only 3% of these investigations recorded that the setts/latrines were fenced off from livestock contact.

Chapter 7 of this dissertation was also part of the ‘chronic Tuberculosis herd project’ and used the definition from Chapter 3 to look at the farm management factors associated with recurrent bTB herd breakdowns (defined as a bTB herd breakdown of duration less than one year followed by two more breakdowns within two years). This study reported that the main source of infection driving recurrent bTB herd breakdowns was carryover of infection, most likely caused by failure of diagnostic tests to clear herd infection during the breakdown period. It also provided insight into the role of herd type and herd size as confounding factors in recurrent breakdown situations.

General Discussion

The Northern Ireland chronic Tuberculosis herd project

Griffin et al. were among the first authors to publish work investigating the chronic nature of bTB at herd level and became part of an international effort to determine likely associated risk factors [3]. This observation was borne out from experience in the ROI where the bTB eradication programme began in 1954 and, using a “test and slaughter” policy herd, incidence had reduced from 80% to 2.8% by 1965. However this rapid progress towards eradication was not maintained and incidence levels in 1988 were only slightly below 1965 levels [3, 4]. This pattern of dramatic initial success in bTB control leading to different forms of intractable situations is typical for most countries of the British Isles [5]. These intractable states are well described in the “TB TRENDS” papers [2, 6] (Chapter 7) but are summarised as a general increasing incidence from the late 1980s in England, Wales and NI while in ROI, bTB presents as an ongoing problem which has reduced to a low level endemic situation.

Spotlight on the problem of chronic bTB herds in NI was formally raised by Abernethy and Menzies in 2005 through an internal government report [7]. This report showed that between 1995-2004 herds having had at least three bTB herd tests where SICCT test reactors were disclosed in the previous two consecutive calendar years contributed to over 50% of the total number of SICCT test reactors. The realisation generated by this report was that unless these herds could be successfully cleared of infection they presented a significant barrier to bTB eradication and, in themselves, provided enough disease output to prevent eradication.

NI was not alone at the time in terms of this thinking as a plethora of research across the British Isles was ongoing in an attempt to understand the risk factors driving chronically infected bTB herds [3, 8-14]; the aim being to use this understanding to provide better control interventions. Much of the research based on how chronically bTB affected herds divided into two general types; persistent infection (long duration breakdown periods) [10, 14] and recurrent infection (recurrent breakdown periods) [3, 11, 13] (or both together [12]).

The main contributors to the phenomenon of chronic bTB herds are the inability of disease control measures to completely remove infection and repeated introduction of infection. Many factors can influence the effectiveness of these two contributing mechanisms and will be the subject of the following discussion.

Herd Size

Three chapters of this dissertation were studies focusing specifically on chronic bTB herds and in each study, herd size was an important explanatory factor (in the recurrence bTB herd breakdowns in Chapter 3 and as interaction with other variables in Chapters 6 and 7). It is no surprise that herd size appears as a consistent explanatory variable in these studies as it has been reported by several other authors researching bTB [3, 9, 12, 13, 15-17]. Despite this plethora of research, an explanation as to why herd size frequently appears as an explanatory variable has never really been untangled. Skuce et al. summarized the position of herd size as a risk factor in a number of previous studies pointing out that it was unclear whether this variable was acting as a risk factor *per se*, or whether it formed a proxy variable as a partial summary measure of other factors. Indeed herd size could be acting to inherently change the herd level sensitivity and specificity of the test, as herd size increases [18]. One important aspect of NI herd size is its correlation with herd type (average herd size; dairy vs beef breeding was 95 and 15 cows, respectively) [19], though the relationship between bTB disclosure and herd size/type is not well defined in the published literature.

In terms of contributing towards chronic bTB infection, herd size is a factor which can both influence the inability to remove infection and the repeated introduction of infection. Given the already stated limitations of diagnostic test sensitivity (SICCT test and Interferon gamma tests), one of the most obvious issues for large herds are false negatives. False negatives contribute to both prolonged and recurrent bTB chronic herd breakdowns by allowing infected animals to remain in the herd, continuing the spread of infection. An observation from Chapter 7 was the impact of the number of land parcels (in a farm holding) on recurrent bTB herd breakdowns. Results showed that as herd size increased those herds with greater than three land parcels had a much weaker association to such bTB breakdowns than those with three or less. This result points to a possible explanation that farmers with several land parcels and a large herd size are more likely to manage their herd as separate operations. This allows more efficient removal of infection from a particular group of cattle, which in terms of diagnostic testing act as a ‘small’ herd. Other factors which may have impact on the ability of control measures to remove infection and which are more likely to have undue effects on larger herds, especially beef fattening units which purchase from numerous sources are concurrent problems with local endemic infectious diseases.

Chapter 6 demonstrated a statistically significant association of prolonged bTB breakdowns to both liver fluke (*Fasciola hepatica*) and IBR (Infectious Bovine Rhinotracheitis). It was found that use of IBR vaccination (used as a proxy for IBR exposure within a herd) increased the odds of developing a prolonged bTB herd breakdown (OR = 1.476). Even though the influence of respiratory infections on the susceptibility to infection with *M. bovis* remains untested, it is speculated that such infections can facilitate increased aerosol spread [18]. With large herds the proportionate influence of respiratory infections could be magnified; especially in the case of beef fattening herds where a lot of cattle can be kept in high density housing with associated stress factors. This in turn leads to ideal conditions for respiratory disease [20], possibly spreading infection at a high rate, negating the effectiveness of control measures.

Herd size has an impact on risk of repeated introduction of infection based on the increased epidemiological contact that large herds have with other herds and their direct environment. Some of these contacts can be mitigated by implementation of better biosecurity measures, such as minimising purchase of cattle, risk assessing purchases and maintaining effective barriers with wildlife. In some herds, such measures are more difficult to implement, such as where the business model involves purchase of cattle. In the study from Chapter 6, herd size had a statistically significant interaction with the source of infection in prolonged bTB herd breakdowns; the most important being purchase of cattle, followed by badgers, both of which become more significant with increasing herd size. Exploring this further showed that prolonged bTB herd breakdowns with purchase as a source were mostly (75%) composed of beef fattening herds, which is probably not surprising, but did demonstrate this factor as the primary driver of chronicity in these herds.

With the knowledge that larger herds have much greater difficulty clearing infection and animals within these herds are likely to be at greater risk from repeat introduction of infection, tailored control measures for larger herds may be necessary. Results from the Chapter 6 study support the hypothesis that good biosecurity, which is important in all herds, may actually have even greater benefits in a larger herd. The practical application of this would be more focus on the biosecurity of large herds, incentivising good practice such as fencing off badger setts/latrines, risk assessing purchases and reducing epidemiological links to other herds at grazing time. In terms of clearing infection, again it may be necessary to look at larger herds in a different light compared to smaller herds. Given that diagnostic testing is less effective in clearing infection from larger herds, it may be necessary to apply longer restrictions and more cycles of diagnostic testing to these herds. Where practical in larger herds, it may also be of benefit to separate them into smaller epidemiological units, while applying more stringent controls on the problem cohorts.

Cattle Movement

As a risk factor for bTB herd breakdowns, movement of infected cattle is an area which has received much investigation [12, 21-26]. These studies have looked at different aspects, such as cattle movements from markets and farm sales [24, 25], cattle purchased from high risk herds [21] and severity of the original bTB herd breakdown [26]. In NI, purchase of beef/store animals from a herd which had a bTB breakdown in the previous three years was shown to be a risk factor, but no quantification of the effect was calculated [27]. Also, genotyping of *M. bovis* has demonstrated that bTB can translocate significant distances from its normal geographical location and can be linked directly to animal movement [28]. The actual contribution cattle purchases make to bTB herd breakdowns has been estimated in ROI (6-7%) and Great Britain (GB) (16%) [22, 23].

Chapter 4 had the objective of quantifying the contribution of officially recorded cattle movements to bTB herd breakdowns on NI farms. With this study, it is important to note that the type of cattle movements assessed were those officially recorded/registered on the national database (APHIS). Cattle movements can be the officially recorded inter-herd movements or, probably the more common, not recorded intra-herd movements, where cattle are moved between units of farms under the same management. After controlling for the infection process occurring in the disclosing bTB breakdown herd, our study showed that 6·4% (95% CI 5·9–6·8) of bTB herd breakdowns in NI were directly attributable to the movement of infected animals. The results (Chapter 4) also indicated that of the 12,060 confirmed bTB herd breakdowns (2007–2015 inclusive), 31% (95% CI 29·8–31·5) contained a confirmed bTB reactor(s) or LRS (Lesion at Routine Slaughter) at the disclosing herd test that had entered the herd within the previous 450 days.

The studies discussed above describe the impact movement has on the cattle herd population as a whole, with results suggesting that it is not the main source in initiation of bTB breakdowns. However, that is not to say cattle movement does not provide a mechanism of repeat introduction of infection into some bTB chronically infected herds. Chapter 6 showed the importance of purchase in driving prolonged bTB herd breakdowns, albeit mainly in beef fattening herds, while recurrent breakdowns (Chapter 7) did not have any significant associations to cattle movement as a source. Beef fattening herds with their purchase policies act as a focal point for disclosure of infected animals and given their nature as generally large intensively run units must also have the conditions necessary to promote intra-herd spread. Potential policies to alleviate it could be information available to purchasers about the risk of infection associated to the cattle they plan to acquire; based on historical data linked to the selling herd. Such information would allow the purchaser to potentially reduce the number of repeat introductions of infection to their herd. Another policy not currently implemented in NI which could help to reduce the movement of infected animals is the SICCT testing of all cattle over 42 days of age before they are allowed to move to another herd, if they have not been tested in the previous 12 months [29].

Wildlife

Mycobacterium bovis infection in cattle and badgers (*Meles meles*) is a persistent and costly problem for the farming industries and governments of the UK and ROI [5]. Prevalence estimate for NI based on the monitoring of road traffic killed badgers was reported to be 15.3% (95% CI: 13.1-17.5%) [30]. Evidence of an association between *M. bovis* infection in cattle and badgers exists from a variety of sources. In ROI the role of the badger in maintaining persistent infection in cattle herds was examined in the east Offaly (1989-1995) and four area (1997-2002) field trials where proactively culled areas were compared to matched reference areas in which reactive culling was carried out [31-34]. In the GB a field trial, the RBCT (Randomized Badger Culling Trial) was carried out between 1998 and 2006 which compared areas of proactive culling to matched areas in which there was no badger culling [35]. The results of both the ROI and GB work showed that levels of bTB in cattle were lower in areas subject to extensive proactive badger culling compared to matched control areas [33]. More recently in the UK (2013-2017), industry led proactive badger culling was assessed for its effects on bTB herd incidence, where licensed culling areas were matched to comparison areas. The results of this work over a four year period showed that there was a reduction in bTB herd incidence in two of the three study areas [36, 37].

Other important evidence which supports an association between *M. bovis* infection of cattle and badgers in a locality is molecular genotyping where UK and Irish studies show that cattle and badgers tend to share the same genotypes in the same areas [38-40].

Wild deer are another potential source of *M. bovis* infection for cattle. Previous work in ROI, England and Wales have identified deer as a potential *M. bovis* source [41-44]. However, a *M. bovis* prevalence survey carried out in NI showed disease levels to be low in deer, *circa* 2% (DAERA, unpublished data). Additionally, NI studies which investigated deer as a risk factor for bTB herd breakdowns found no significant association although it may still be a contributing factor in some very localised areas of NI [27, 45].

With chronically bTB infected herds where a wildlife source is implicated, this could potentially provide repeat introduction of infection. The nature of the transmission of infection from a badger source is reported as a low level introduction of infection into a herd, which is followed by cattle-to-cattle amplification and onward transmission to other cattle herds [46]. Results from Chapters 6 and 7 investigated how source of infection was linked to prolonged and recurrent bTB herd breakdowns. Chapter 6 demonstrated a strong association between beef fattening herds and purchase of cattle as a risk source, but possibly more importantly, it showed that unlike purchase, which was focused on beef fattening herds, badgers were a risk to all herd types. Chapter 7 which reported on recurrent breakdowns determined these to be strongly associated to carryover of infection from a previous breakdown, with other sources such as badgers showing no statistically significant association.

In relation to bTB, cattle and badgers exist in what could be described as a multi host system with bi-directional transmission of infection [47-50]. Current evidence on interactions between badgers and cattle shows that direct contact between the species capable of providing opportunity for aerosol spread of disease is a very rare event [51-54]. Rather fomite transfer in which badgers and cattle use the same space but at different times, may result in the potential transfer of infectious material [53-56]. As badgers are an implicated source for prolonged breakdowns, preventing the introduction of bTB infection into a herd must be a basis for any control measure.

This research (Chapters 6 and 7) found that almost one-third (30%) of farms investigated with bTB herd breakdowns were recorded as having badger setts, but only 3% of these investigations reported farms where badger setts and/or latrines were fenced off. This result shows that on NI bTB breakdown farms there is relatively little biosecurity barrier for fomite transfer of infection between badgers and cattle; a result consistent with previous work [27]. It must also be noted that the two host bTB transmission system between badgers and cattle is a complex interaction probably varying between different localities, which is dependent on a range of badger and cattle metrics unique to a particular area [47-49]. For measures to have real impact upon the infection transmission between badgers and cattle (and *vice versa*) they must be applied in tandem to both hosts [48]. In terms of attempting to control bTB introductions from badgers into NI herds, disrupting the fomite transfer of infection is a biosecurity issue which must be addressed. The advantage of applying such measures, for example fencing off setts and latrines in a biosecure way has impact on both cattle to badger and badger to cattle transmission. Indeed it is likely that if the present level of biosecurity reported in this dissertation is not improved, any other control measures applied to the badger/cattle transmission system will be of limited impact.

Inter-herd contiguous spread

The first NI publication which attempted to put an aetiological fraction on the importance of cattle to cattle contiguous herd spread estimated this value at 40% [45]. This 1999 study also stated that 79% of fences in NI did not prevent nose to nose contact between herds [45]. In a more recent NI study carried out in 2016, contact between neighbouring cattle was assessed as possible through 66.8% of boundaries, however no significant association was found between boundary contact and bTB herd breakdowns [27]. Other NI research which studied grazing cattle exposure to neighbouring herds and badgers in relation to bTB risk found that 89% of farms grazed cattle adjacent to a neighbouring herd, accounting for 18% of the grazing season, though in this work historic bTB status and percentage of days spent beside a neighbouring herd was unrelated [51]. Irish work that investigated the relative importance of 'neighbourhood' (other local sources and residual infection) on bTB persistence found that bTB incidence was associated with an increased animal incidence in two subsets of neighbouring herds: (1) herds directly contiguous during the previous 2 years (attributable fraction = 0.20), and (2) herds at a distance of >25 m in the previous year (attributable fraction = 0.19) [14].

In NI, cattle are generally housed during the winter season and grazed on pasture during the rest of the year. Subject to weather conditions and grass growth, there are variations on when cattle are removed from pasture in the autumn and returned to grazing in the spring. Farms in NI are historically fragmented in nature leading to a high degree of boundary contact and ample conditions for contact between herds [8]. Given the relative ease by which cattle excreting droplet nuclei containing *M. bovis* bacteria can infect other cattle [57, 58], contact between neighbouring herds must present a risk, leading to the potential for repeat introduction of infection through this route.

Chapter 6, which looked specifically at prolonged bTB herd breakdowns, found that either recent upgrading or complete installation of new boundary fences showed a significant negative association with the duration of bTB herd breakdowns. This result provides circumstantial evidence for the application of better biosecurity measures in the form of adequate boundary fences to reduce cattle-to-cattle contiguous herd contact could reduce the odds of a prolonged bTB herd breakdown. Results from Chapter 7 were less equivocal about the benefit of fixing or upgrading fences in order to reduce the association with recurrent bTB herd breakdowns. This could be explained from the point of view that the source of infection for recurrent bTB herd breakdowns (Chapter 7) was strongly associated to carry over of infection. In these situations where carryover was the dominant source, the state of fencing may not have a large bearing on the course of a breakdown as neighbours may be of less importance to these cases.

Other variables found (Chapter 7) to be associated to recurrent bTB herd breakdowns were the practice of grazing cattle on ground after silage removal and situations where the farmer highlighted parcels of land they considered to be of higher bTB risk to their cattle. However, it was pointed out in Chapter 7 that these factors could represent risk from a mixed source. In the case of grazing cattle on ground after silage removal, this practice forces cattle to forage around field perimeters thus in closer proximity to contiguous herds [51]. Additionally, probably increasing exposure to badger setts and latrines which are more likely to be located in hedgerows in a NI setting [27, 59-61] and moreover, very unlikely to be fenced off.

Prevention of introduction of bTB infection, through contiguous spread between herds is a biosecurity issue with control principles based on placing an effective barrier between herds. Indeed this source of infection introduction has been realised for many decades, with little evidence of the necessary biosecurity improvements to date. Many farmers in NI also supplement their holdings by renting extra ground (conacre). One of the disadvantages to this system is that rented ground can suffer from a general underinvestment, especially in the quality of fencing present, possibly exacerbating problems of inter herd contact.

Inability of control measures to effectively remove herd infection

Cattle are a maintenance host for bTB, which means the cycle of infection can be sustained in them without any other host interaction [20, 62]. The NI bTB eradication programme (as described in the introduction to this dissertation) are a combination of ante and post-mortem tests designed to constantly detect and remove bTB infection from cattle. Successful eradication schemes for bTB have been implemented in a number of European and other countries over the past 50 years using similar approaches to NI [5, 63].

Alongside processes which introduce infection into a herd, bTB can also be maintained due to the inadequacy of the diagnostic tests. If a herd repeatedly fails diagnostic tests, they remain OTW restricted (prolonged bTB herd breakdowns – Chapters 4 and 6). However, if an OTW herd passes the required statutory tests it will regain its Officially Tuberculosis Free (OTF) status (allowed to commence inter-herd trading of animals); but if it is not cleared of infection or becomes re-infected, it will disclose bTB at some future surveillance test (recurrent bTB herd breakdowns – Chapters 4 and 7). Undetected infection is likely be present in both prolonged and recurrent bTB herd breakdowns and is well recognised in the literature [11, 64-67]. In recurrent bTB herd breakdown situations where derestriction following statutory testing is achieved, persistent undetected infection would be expected to lead to more rapid disclosure than re-infection from other sources [15]. Even though undetected infection is likely to have a role in prolonged bTB herd breakdowns (main source risks here are reinfection events – Chapter 6), results from Chapter 7 point to it being the main source for recurrent bTB herd breakdowns.

At a national level, the practical implications of bTB herd breakdown recurrence at six monthly and yearly post breakdown herd tests (measured as a percentage of the tests carried out in the previous year) was 11% and 9% disclosing infection, respectively. These figures are similar to those for herd derestriction tests (reinstating OTF status to a herd), at 11% (DAERA 2021 unpublished data); this figure only being surpassed by herd tests carried out during bTB herd breakdown events, demonstrating the probable importance of carryover of infection.

As diagnostic testing is a fundamental control measure to the NI bTB programme, obvious failures in its effectiveness to remove infection are a serious demoralising factor for both the farmers and vets involved. When infection is confirmed in a herd, it must have two consecutive tests at least 60 days apart at which no test positive animals are disclosed; which along with cleansing and disinfection of the farm allows the herd to be derestricted and off farm trade to commence. Given the level of breakdown recurrence in NI, it must be the case in some herds two consecutive tests at 60 days apart are not enough to remove all infection, probably not surprising given the test characteristics of the SICCT test. The SICCT test has a high average specificity (99%-100%) but a relatively modest average sensitivity (51%-80%) at standard interpretation [68]. Within the NI bTB programme, the Interferon gamma test is applied in parallel with the SICCT test. This parallel application to herds with proven disease is implemented so as to detect and remove more infected animals. The Interferon gamma test has better sensitivity (88-94%), but poorer specificity (85-98%) than the SICCT test, making it more likely to disclose false positives [68]. However counter to measures which aim to increase diagnostic test sensitivity, its reduction can be brought about by repeated short interval testing such as that which occurs in chronically infected herds [69] and co-infection with liver fluke; this acting to mask the true bTB status of animals, making clearance of infection from the herd more difficult [5]. Results from Chapter 6 show that application of fluke treatment was significantly associated with a reduced odds of developing a prolonged bTB herd breakdown with the hypothesis being that a properly fluke treated herd allows better functioning of the SICCT test and thus more efficient clearing of infection.

Another factor which has important bearing on the effectiveness of control measures to remove herd infection is the number of diseased animals present in a herd. Along with results from Chapters 2 and 3 of this dissertation, other publications demonstrate a link between residual infection and the number of SICCT test reactors disclosed during a bTB herd breakdown [13, 17, 70]. Policy changes to take account of this work have been made to the NI bTB programme, such as in March 2018 where herds had their OTF status removed and replaced with OTW when more than one SICCT test reactor disclosed during the course of a bTB herd breakdown as opposed to more than five SICCT test reactors previously required to obtain OTW status. Policies such as this aim to apply control measures in a more stringent way, thus attempting to reduce the maintenance ability of infection in a herd. However it is probable that on their own even severe application of control measures which focus on removal of infection from a herd will not be enough to eliminate chronic bTB herd breakdowns and additional measures which control the flow of infection to and from herds will also need to be implemented.

Recommendations

Recommendations to address chronic bTB control measures must impact on the inability to remove and reduce repeated introduction of infection to herds.

It only takes infrequent, repeated introduction to maintain infection in a herd. For this reason, without a herd biosecurity barrier any measure aimed at controlling infection in sources such as wildlife is likely to be of limited effect. The types of intervention which can have most impact are those which limit contact between badger setts/latrines and cattle where there is potential for fomite spread of infection. The formation of a biosecure area around badger setts/latrines is an area which probably needs investigation to determine why it suffers from such poor compliance by herd keepers. One reason as to why there is such a low percentage of setts/latrines fenced off in bTB infected herds may be that farmers receive mixed messages from government. This mixed message comes from the disease control and subsidy payment sections of DAERA; with disease control served best by wiring off areas of fields containing setts/latrines while subsidy is based on maintaining the historical area of the same fields. In terms of a broader recommendation for government, any introduction of agricultural/environmental support schemes must include a statement demonstrating its consideration of impact on and promotion of biosecurity, at all times aiming for best practice [71].

Another area where repeat introduction of infection leads to mainly prolonged bTB herd breakdowns is the purchase of bTB infected animals. Recommendations to control this source could include information provision to purchasers about the risk posed by the animals they intend to buy or using more stringent surveillance to prevent the movement of infected animals. Currently in the NI bTB programme, any individual animal which has not been tested in 15 months will have their movement restricted, which does not prevent the purchase of infected animals. A more effective system which is applied in the ROI is to prevent the movement of cattle from their present herd if they have not been SICCT tested in the previous 12 months.

The size of herds in NI has continuously increased over time and results from this dissertation and other published works provide evidence for its role in both prolonged and recurrent bTB herd breakdowns. Thus for large herds it may be necessary to introduce specifically tailored controls. These could include the routine use of more sensitive diagnostic testing and possibly more focus on concurrent endemic diseases in these herds. In terms of introducing specific controls for large herds, work is still required to define in what circumstances they should apply. Where it is practical, it may be possible to look on large herds as separate epidemiological units applying different controls based on risk from each separate cohort of the herd, though this type of approach requires high levels of cooperation from the herd keeper. On a broader point, it may be that within the NI agricultural environment that chronic bTB is just a price that has to be paid to maintain ever larger herds, which are attractive based on economy of scale but maybe not taking full account of disease costs.

Ability to efficiently remove infection from chronic bTB herd breakdowns is an essential component of the NI eradication programme. This is an area which requires further research, such as a better understanding of diagnostic test performance in bTB herd breakdowns and further investigation into why some infected animals are not detected, being left to maintain the herd in a diseased state. One important risk factor for herd breakdowns is the number of disclosing SICCT reactors and its relationship to recurrence of future breakdowns. With situations where there are large numbers of disclosing SICCT reactors, it may be the case that these herds require longer restricted periods and more herd tests to remove residual infection and limit their ability to trade with OTF herds. However introduction of a policy change of this nature would require more research to determine its potential effectiveness.

Conclusion

Northern Ireland has a comprehensive bTB programme which faces many challenges. The main subject of this dissertation, chronic bTB herds, is one such example, bringing together many factors which interact in a complex manner providing a difficult backdrop in which to apply control policies. The challenges faced include insufficiencies in diagnostic techniques, programme fatigue among farmers who feel a lack of empowerment about their circumstances and an agricultural industry evolving in ways which potentially make disease control more difficult. However, even though the challenges are great it will only be through logical and well thought out science, including social science, that bTB eradication can be achieved.

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Chapter 9

Summary

Summary

Bovine tuberculosis (bTB) is an infectious disease of cattle that affects practically all vertebrates and is caused by acid-fast bacilli from the genus Mycobacterium, most notably *M. bovis*. It has a global distribution and classically is defined as a chronic debilitating disease; however it occasionally assumes an acute and rapidly progressive course. There are several ways in which bTB spreads between cattle including horizontal spread by inhalation or ingestion and, less commonly by vertical transmission. It is however widely accepted that aerosol is the most important infection route with very low numbers of bacteria required for establishing infection. In Northern Ireland (NI), first statutory attempts at control of bTB began in 1949 with a compulsory programme introduced in 1959. However, despite the current national bTB eradication programme of annual test surveillance along with enhanced risk testing (with removal of test positive animals from the population, curtailing infection transmission) and abattoir surveillance, bTB has remained endemic in the NI cattle population. *Mycobacterium bovis* is a zoonosis, but is relatively rare found in humans within the EU (European Union). This contrasts with developing countries where *M. bovis* infection may still constitute a major threat to public health, with the fraction attributable to human TB cases largely unknown, but likely to be much higher than in industrialised countries and speculated to account for as many as 10-15% of new human cases.

The subject area addressed in this dissertation was the epidemiological investigation of chronic bTB herd breakdowns in NI. Chronic bTB herd breakdowns have received much scientific investigation across the countries of the British Isles and beyond. Initial epidemiological analysis from a NI context originated in a 2005 paper which demonstrated the importance of these herds in a local context, directly leading on to establishment of the "chronic bTB herds project" on which this dissertation is based. As described in the dissertation, the main contributors to the phenomenon of chronic bTB herds are the inability of disease control measures to completely remove infection and repeated introduction of infection. Many factors can influence the effectiveness of these two contributing mechanisms, with the discussion in this dissertation based on the following areas:

1. Herd size
2. Cattle movement
3. Wildlife
4. Inter-herd contiguous spread
5. Inability of control measures to effectively remove herd infection

In terms of contributing towards chronic bTB infection, herd size is a factor which can both influence the inability to remove infection and the repeated introduction of infection. Discussed in the dissertation was the impact of diagnostic test sensitivity (SICCT test and Interferon gamma tests). One of the most obvious issues for large herds are false negatives, which contribute to both prolonged and recurrent bTB chronic herd breakdowns by allowing infected animals to remain in the herd, continuing the spread of infection. Herd size has an impact on risk of repeated introduction of infection based on the increased epidemiological contact that large herds have with other herds and their direct environment. Chapter 6 highlights a statistically significant interaction between herd size and source of infection in prolonged bTB herd breakdowns; the most important being purchase of cattle. Exploring this further showed that prolonged bTB herd breakdowns with purchase of cattle as a source were mostly (75%) composed of beef fattening herds, demonstrating this factor as the primary driver of

chronicity in these herds. Chapter 4 looked directly at how official cattle movements in NI impact on disclosures of bTB herd breakdowns and showed that 6·4% (95% CI 5·9–6·8) of bTB herd breakdowns in NI were directly attributable to the movement of infected animals. This result would tend to suggest movement of animals is not a major source in initiation of bTB herd breakdowns; however that is not to say cattle movement does not provide a mechanism of repeat introduction of infection into some bTB chronically infected herds. Chapter 6 and 7 investigated how wildlife impact on chronic bTB herd breakdowns. Chapter 6 demonstrated a strong association between beef fattening herds and purchase of cattle as a risk source, but possibly more importantly, it showed that unlike cattle purchases, which was focused on beef fattening herds, badgers were a risk to all herd types. Chapter 7 which reported on recurrent bTB herd breakdowns determined these to be strongly associated to carryover of infection from a previous bTB breakdown, with other sources such as badgers showing no statistically significant association.

Another important result was that almost one-third (30%) of farms investigated with bTB herd breakdowns were recorded as having badger setts, but only 3% of these investigations reported farms where badger setts and/or latrines were fenced off. This result shows that on NI bTB breakdown farms there is relatively little biosecurity barrier for transfer of infection between badgers and cattle.

With inter-herd contiguous spread of bTB, Chapter 6 looked specifically at prolonged bTB herd breakdowns. It found that either recent upgrading or complete installation of new boundary fences showed a significant negative association with the duration of bTB herd breakdowns. This result provides circumstantial evidence for the application of better biosecurity measures in the form of adequate boundary fences to reduce cattle-to-cattle contiguous herd contact could reduce the odds of a prolonged bTB herd breakdown.

Alongside processes which introduce infection into a herd, bTB can also be maintained due to the inadequacy of the diagnostic tests to fully remove infection from a herd. This inability of control measures to effectively remove infection was described as the main driver of recurrent bTB herd breakdowns (Chapter 7). As diagnostic testing is a fundamental control measure to the NI bTB programme, obvious failures in its effectiveness to remove infection are a serious demoralising factor for both the farmers and vets involved. This is reflected at a national level as bTB herd breakdown recurrence at the six monthly and yearly post breakdown herd tests (measured as a percentage of the tests carried out in the previous year) with herd disclosure levels at 11% and 9%, respectively.

Recommendations to address chronic bTB herds were made in this dissertation and focused on the key areas of the inability to remove and reduce repeated introduction of infection to herds. These recommendations include increasing farm biosecurity where measures need to be taken to reduce contact between badger setts/latrines and cattle, reducing potential for indirect spread of infection. Another area where repeat introduction of infection leads to mainly prolonged bTB herd breakdowns is the purchase of bTB infected animals. Recommendations to control this source could include information provision to purchasers about the risk posed by the animals they intend to buy or using more stringent surveillance to prevent the movement of infected animals.

The size of herds in NI has continuously increased over time and results from this dissertation and other published works provide evidence for its role in both prolonged and recurrent bTB herd breakdowns. Thus for large herds it may be necessary to introduce specifically tailored bespoke controls. These could include the routine use of more sensitive diagnostic testing and possibly more focus on concurrent endemic diseases in these herds. One important risk factor for bTB herd breakdowns is the number of disclosing Single Intradermal Comparative Cervical Tuberculin (SICCT) reactors and its relationship to recurrence of future bTB herd breakdowns. In situations where there are large numbers of disclosing SICCT reactors, it may be the case that these herds require longer restricted periods and more herd tests to remove residual infection and limit their ability to trade with herds that are bTB free.

In conclusion, it will be very difficult to eradicate bTB from NI cattle herds without logical and well thought out disease eradication policies that utilise a science evidence base, which should embrace multiple scientific disciplines including social science.

Chapter 10

Samenvatting

Samenvatting

Runder tuberculosis (TB) is een besmettelijke ziekte voor runderen, maar het kan ook andere vertebraten aantasten. Het wordt veroorzaakt door zuurvaste baccillen van het genus Mycobacterium, met name *M. bovis.* De distributie is wereldwijd en het is gedefineerd als een chronische, slopende ziekte; alhoewel het soms ook een snel en acuut verloop heeft. Er zijn verschillende manieren waarop TB zich verspreid tussen runderen zoals horizontaal, via inhalatie en inslikken, en ook, maar minder vaak, via verticale transmissie. Het is algemeen aanvaard dat aerosol de meest belangerijke infectie route is en dat er slechts enkele bacterien nodig zijn om een infectie te veroorzaken. In Noord-Ierland (NI), de eerste pogingen om TB onder controle te krijgen begonnen in 1949 en een verplicht controle programma was geintroduceerd in 1959. Het huidige nationale TB eradicatie programma is gebaseerd op jaarlijks testen en meer frequent testen van risico kuddes (met het verwijderen van test positieve dieren uit de populatie om het verspreiden van infectie te verminderen) en toezicht in het slachthuis, maar TB is nog steeds endemisch in de runder population in NI. *Mycobacterium bovis* is een zoonose die relatief zeldzaam is in de EU (Europese Unie). Dit is het tegenovergestelde in onderontwikkelde landen waar infecties met *M. bovis* nog steeds een grote bedreiging zijn voor de volksgezondheid en waar het gedeelte dat bijdraagt aan TB infecties in mensen nog steeds onbekend is maar waarschijnlijk veel hoger ligt dan in geindustraliseerde gebieden en gespeculeerd is bij te dragen tot 10-15% van de nieuwe infecties in mensen.

Het onderwerp van deze dissertatie is het epidemiologisch onderzoek dat is gericht op kuddes met chronische TB besmettingen in NI. Er is al veel onderzoek gedaan naar chronische TB besmettingen, niet alleen in het Verenigd Koninkrijk en Ierland, maar ook in andere landen. Het eerste epidemiologisch onderzoek in NI op dit gebied dateert terug naar 2005 en toont het belang van deze chronische besmette kuddes aan. Dit leidde naar het "chronische TB project" waarop deze dissertatie is gebaseerd. Deze dissertatie beschrijft de belangerijkste factoren die bijdragen aan chronische TB besmettingen in kuddes. Deze factoren zijn het onvermogen van de huidige ziekte control methodes om de besmetting geheel te verwijderen en de herhaalde introductie van nieuwe besmettingen. Veel factoren kunnen de effectiviteit van deze twee mechanismen beinvloeden en dit is waar de discussie in deze dissertatie op is gebaseerd:

1. Hoeveel runderen er in de kudde zijn
2. Runderen die van de ene kudde naar de andere kudde gaan
3. Wilde dieren
4. Verspreiding van besmettingen tussen kuddes die dichtbij elkaar liggen
5. Het onvermogen van controlemaatregelen om effectief besmetting te verwijderen uit een kudde

Hoeveel runderen er in een kudde zijn heeft een belangerijke invloed op het ontwikkelen van een chronische TB infectie, omdat het een invloed kan hebben op het onvermogen om de besmettingsbron te verwijderen en het risico dat besmettingen steeds weer terug komen. De invloed van de sensitiviteit van de diagnostische testen (SICCT test en gamma interferon test) wordt geevalueerd in deze dissertatie. Het aanwezig zijn van test negatieve, besmette runderen is een van de meest voor de hand liggende redenen voor grote kuddes om langdurige en herhaalde chronische TB besmettingen te hebben, omdat deze besmette dieren in de kudde blijven en zo de infectie verspreiden. Hoeveel runderen er in een kudde zijn beinvloedt het risico van herhaalde introductie van infectie gebaseerd op het feit dat grote kuddes meer contact hebben met andere kuddes en hun directe omgeving. Hoofdstuk 6 maakt duidelijk dat er een statistische significante interactie is tussen het aantal runderen in een kudde en het inkopen van runderen. Het toont aan dat het inkopen van runderen de belangerijkste infectiebron voor langdurige TB besmettingen is. Als we in meer detail hier naar kijken kunnen we zien dat de kuddes met langdurige TB besmettingen veroorzaakt door het inkopen van runderen met name kuddes met vleesrunderen zijn (75%). Hoofdstuk 4 richt zich op runderen die van de ene naar de andere kudde gaan in NI en hoe deze een effect hebben op TB besmettingen. Dit hoofdstuk toont aan dat 6.4% (95% CI 5.9-6.8) van de kuddes met TB besmettingen in NI worden veroorzaakt door deze factor. Dit resultaat toont aan dat runderen die van de ene naar de andere kudde gaan geen belangerijke besmettingsbron zijn voor een TB infectie in een kudde. Dit betekent alhoewel niet dat het inkopen van runderen geen bron is voor herhaalde besmettingen in sommige chronische geinfecteerde kuddes. Hoodstuk 6 and 7 bekijken hoe wilde dieren een invloed hebben op kuddes met een chronische TB infectie. Hoofdstuk 6 demonstreert dat er een sterke associatie is tussen kuddes met vleesrunderen en het inkopen van runderen als een risico factor. Het toont ook aan dat in tegenstelling tot het inkopen van runderen, met name in vleesrunder bedrijven, dassen een risico factor zijn voor TB besmettingen. Hoofdstuk 7 richt zich op kuddes met zich herhalende TB besmettingen en toont aan dat het overdragen van infectie van een infectie bron die eerst aanwezig was erg belangerijk is en andere infectie bronnen, zoals dassen, niet.

Een ander belangerijk resultaat was dat bijna een derde (30%) van de boerderijen die met TB zijn besmet dassen hollen hebben waarvan slechts 3% ontoegangelijk zijn gemaakt voor runderen door de veehouders. Dit resultaat toont aan dat in NI de kuddes met een TB besmetting over het algemeen niet veel maatregelen nemen om het overdragen van een besmetting tussen dassen en runderen te voorkomen.

Hoodstuk 6 richt zich op het verspreiden van TB tussen kuddes, met name ten opzichte van langdurige TB besmettingen. Het is aangetoond dat nieuwe of goed geinstalleerde omheiningen een negatieve associatie hebben met de duur van de TB besmetting. Dit resultaat toont aan dat een betere omheining het contact tussen runderen vermindert en dat zo ook het risico op een langdurige TB infectie vermindert.

Een nieuwe infectie kan worden geintroduceerd in een kudde, maar TB kan ook in een kudde blijven doordat de diagnostische testen niet voldoende werken om de infectie van de kudde te verwijderen. De inefficientie van controle maatregelen on infectie te verwijderen is een van de belangerijkste factoren dat TB besmetting zich herhaald in kuddes voordoen (hoofdstuk 7). Het onvermogen om infectie te verwijderen is een demoraliserende factor voor veehouders en dierenartsen, omdat diagnostische testen een fundamenteel onderdeel zijn van het Noord Ierse TB programma. Dit is duidelijk op het nationale niveau omdat 11% van de 6 maanden test and 9% van de jaarlijkse test na een infectie positief is.

Aanbevelingingen om chronische geinfecteerde kuddes aan te pakken zijn beschreven in deze dissertatie en zijn met name gericht op het onvermogen om infectie te verwijderen en het verminderen van introductie van infectie in een kudde. Deze aanbevelingen zijn gebaseerd op maatregelen om besmetting te voorkomen, met name in relatie tot het verminderen van het contact tussen runderen en dassen hollen zodat indirecte verspreiding van infectie verminderd is. Aanbevelingen om deze besmettingsbron onder controle te houden kan gebaseerd zijn op het verschaffen van informatie over het risico kopers van runderen lopen en strengere maatregelen zodat geinfecteerde dieren niet van de ene kudde naar de andere gaan.

De grootte van de kuddes in NI is toegenomen en de resultaten van deze dissertatie en andere publicaties zijn het bewijs dat dit een rol speelt in zowel langdurige als herhaalde TB infecties in kuddes. Dus voor grotere kuddes kan het nodig zijn om specifieke controles te hebben. Dit kan gebaseerd zijn op het gebruiken van sensitievere testen op een routinematige manier en ook om de aandacht te richten op andere endemische ziektes die meespelen in dezelfde kudde. Een belangerijk risico voor TB infecties is hoeveel test positieve runderen er zijn en her verband tussen dit en TB infecties in de toekomst. Als er veel test positieve dieren zijn dan kan het mogelijk zijn dat deze kuddes langer gesloten moeten blijven ten opzichte van het inkopen en verkopen van runderen en dat ze vaker moeten worden getest om verdere infectie verspreiding te limiteren en te voorkomen dat geinfecteerde runderen worden verkocht naar een kudde zonder TB.

Ten slotte, het is moeilijk om TB uit de Noord Ierse runder populatie te verwijderen en daarom is het belangerijk om goed doordachte methodes gebaseerd op wetenschap toe te passen. Dit wetenschappelijk bewijs moet ook sociale wetenschappen omvatten.

Chapter 11

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Curriculum Vitae

Publications

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Curriculum Vitae

Liam Doyle was born on 17th of September 1971 and is from the townland of Creggan, County Tyrone in Northern Ireland. He received his secondary education at the Christian Brothers Grammar school in Omagh, County Tyrone and thereafter attended the Faculty of Veterinary Medicine of University College Dublin. After graduation he worked as a veterinary practitioner in mixed practice in County Armagh. In 2001 he joined Veterinary Service and took up a post in Enniskillen Campus at the College of Agriculture and Rural Enterprise (CAFRE) teaching both agriculture and equine students at the college. After finishing work placements in local Veterinary offices and a period in the Contingency Planning Unit for Epizootic Disease he joined the Veterinary Epidemiology Unit in 2013. In this current role his main focus is bovine tuberculosis research alongside epizootic disease duties and management of disease based data sets vital to production of quality assured epidemiological outputs.

Alongside a degree in veterinary medicine from University College Dublin Liam holds an MSc in Veterinary Epidemiology and Public Health from University of London, a BSc (Hons) in Mathematical science from the Open University, a BA in Computer Science from the Open University, a certificate in communications from Loughry College CAFRE and a certificate in Machine Learning Applications from the London School of Economics.

Publications

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