Research in Veterinary Science 145 (2022) 205–212

Contents lists available at ScienceDirect

Research in Veterinary Science

journal homepage: www.elsevier.com/locate/rvsc

Evaluating the risk of bovine tuberculosis posed by standard inconclusive reactors identified at backward-traced herd tests in Northern Ireland that disclosed no reactors

Anastasia Georgaki [a], Hannah Bishop [a,*], Alan Gordon [b], Liam Doyle [a], Maria O'Hagan [a], Emily Courcier [a], Fraser Menzies [a]

[a] *Veterinary Epidemiology Unit, Department of Agriculture, Environment and Rural Affairs (DAERA), Dundonald House, Upper Newtownards Road, Ballymiscaw, Belfast BT4 3SB, UK*
[b] *Agri-food & Biosciences Institute (AFBI), 18A Newforge Lane, Belfast BT9 5PX, UK*

ARTICLE INFO

Keywords:
Bovine
Tuberculosis
Backward trace
Inconclusive reactor

ABSTRACT

Bovine tuberculosis is a notifiable disease in Northern Ireland with the national eradication programme of compulsory testing and slaughter of reactor animals costing approximately £40 million per year. Backward tracing, known as Backward Check Tests (BCTs), of reactor animals is used to identify previous herds where the bTB positive animal has resided. The aim of this study was to quantify the bovine tuberculosis (bTB) risk posed by inconclusive reactors (ICs) at BCTs at both the individual animal and the herd level. ICs to the Comparative Intradermal Tuberculin Test (CITT) at a BCT, in which no reactors were found, were matched with CITT negative animals, based on age, sex, test ID and follow up period, in Northern Ireland between 1st January 2008 and 31st December 2017 (inclusive). A retrospective matched cohort study design was used with the outcome of interest being the bTB status of each animal and subsequent bTB herd breakdowns. After adjusting for herd size, IC animals at a BCT had 16 times the odds (95% confidence interval: 7.75 to 38.28, $p < 0.001$) of becoming bTB positive compared to CITT negative animals. The percentage population attributable risk was 0.0001%. The majority 75% ($n = 71$) of ICs that became bTB positive were identified at the 42 day retest. Of those that were not disclosed at the 42 day retest ($n = 24$), almost a third (29%) had moved to an unrestricted herd. However, after adjusting for herd size and type, herds that had ICs only identified at a BCT did not have an increased odds of a subsequent bTB herd breakdown compared to herds that had a CITT negative BCT. Given the increased risk posed by ICs at a BCT, it may be justifiable to remove them from the herd immediately or place them under lifetime movement restrictions to the herd where they were detected. However, further action regarding the herd of origin does not appear to be justified.

1. Introduction

Bovine tuberculosis (bTB) is an economically important bacterial infection of cattle, with zoonotic potential, in the United Kingdom (UK) and Ireland (Godfray et al., 2013; More, 2009; More and Good, 2015; Sheridan, 2011). Northern Ireland's eradication programme costs approximately £40 million per year, which poses a significant drain on public finances (NIAO, 2018). During 2020, 1.7 million animals and 22,058 herds were tested disclosing a bTB herd incidence of 8.44% and a bTB animal incidence of 0.747% (DAERA, 2021).

The epidemiology of bTB in the UK and Ireland is complex. There are many potential risk factors that hinder the successful eradication of bTB, including diagnostic test challenges, cattle demographics, wildlife reservoirs and concurrent endemic infections (Broughan et al., 2016; Campbell et al., 2020; Orton et al., 2018; Sedighi and Varga, 2021; Skuce et al., 2011, 2012).

The comparative intradermal tuberculin test (CITT) is regarded as the definitive indicator of infection with *Mycobacterium bovis* (OIE, 2018) and is a central component of the surveillance and eradication programme in Northern Ireland. The technique and its interpretation are described in Annex A of Council Directive 64/432/EEC. The CITT, at standard interpretation, has a high specificity and moderate sensitivity

* Corresponding author.
E-mail address: Hannah.Bishop@daera-ni.gov.uk (H. Bishop).

https://doi.org/10.1016/j.rvsc.2022.01.019
Received 4 November 2021; Received in revised form 20 January 2022; Accepted 24 January 2022
Available online 29 January 2022

(Lahuerta-Marin et al., 2018; O'Hagan et al., 2019). If the bovine reaction is 4 mm greater than the avian reaction, the test is considered positive and, if the bovine reaction is greater than 2 mm and between 1 and 4 mm greater than the avian reaction, the test is considered inconclusive (IC) (DAERA, 2018). Animals with positive CITT test results undergo a detailed post-mortem examination and, if present, bovine tuberculosis-like lesions may be examined histologically and bacteriologically. Animals with positive results at any two of these tests are considered confirmed bTB cases.

A bTB positive animal may have become infected in a previous herd and brought bTB into the current herd where it was detected. Backward tracing is used to identify previous herds where bTB positive animals have resided. If in these herds no CITT test has been carried out since the bTB positive animal moved out, immediate movement restrictions are applied and a Backward Check Test (BCT) of the herd is instigated. All CITT reactors and cattle with bTB lesions at routine slaughter (LRS) are backward traced throughout the course of a bTB herd breakdown. BCTs are high risk herd tests and frequently identify further infection (DAERA, 2018).

It is stated in DAERA's current bTB programme guidelines that when a BCT test identifies an IC animal but no positive CITT reactors then the IC animal(s) is restricted on the farm and it is re-tested approximately 42 days later.

In general, IC animals are known to pose a greater bTB risk than CITT negative animals (Brunton et al., 2018; Clegg et al., 2011a, 2011b; May et al., 2019) but these previous studies have focused on ICs at routine herd testing. The risk of ICs at high risk tests, such as BCTs, is unknown. If the risk posed by ICs is different depending on test type, then the policy for dealing with ICs should differ depending on the disclosing test. This study evaluated the risk of an IC at a BCT (in which no CITT reactors were disclosed) becoming bTB positive at subsequent tests and the risk of the herd of origin having a future bTB herd breakdown.

2. Methods

A retrospective, cohort study was conducted. The dataframe included BCTs conducted between 1st January 2008 and 31st December 2017 (inclusive) in Northern Ireland which disclosed one or more ICs but no CITT reactors. A backward check test was defined as a test initiated due to backward tracing of all CITT reactor and LRS animal movements during the course of an Officially Tuberculosis Withdrawn (OTW) breakdown. An IC was defined as a bovine reaction greater than 2 mm and between 1 and 4 mm greater than the avian reaction.

Data were extracted from Northern Ireland's national cattle database called the Animal and Public Health Information System (APHIS), which contains animal, herd and movement information as well as each individual animal and herd bTB testing history (Houston, 2001). Data management and statistical analysis were completed using Microsoft ACCESS (Microsoft Corporation, Redmund, WA, USA), MS EXCEL (Microsoft Corporation, Redmund, WA, USA) and R software 4.0.5 (The R Foundation for Statistical Computing, https://www.r-project.org/), including packages tidyverse 1.3.1 (Wickham et al., 2019), lme4 1.1–26 (Bates et al., 2015), lmtest 0.9–38 (Zeileis and Hothorn, 2002), and MuMIn 1.43.17 (Barton, 2020).

Analyses were conducted at both the animal and herd level.

2.1. Animal level methodology

2.1.1. Eligibility and matching criteria

The risk factor being investigated was 'being an IC at a BCT which had no CITT reactors'. All ICs at BCTs in Northern Ireland between the aforementioned dates formed the pool of potential participants for the exposed cohort group. Animals that were still alive at the time of the data extraction were excluded from the study to ensure that all animals had been CITT and post mortem examined at slaughter. If a herd had more than one IC at the BCT, all ICs were included. For brevity, these animals are referred to as IC animals for the remainder of the article.

All CITT negative animals at standard interpretation at BCTs between the above dates formed the pool of potential participants for the Unexposed cohort group. For the remainder of the article, these animals are referred to as CITT negative.

To control for potential confounding factors, in particular those that cannot be measured, such as farm management and exposure to wildlife, eligible animals were matched by sex, age, test ID and follow up period. Matching on test ID meant that matched IC animals and CITT negative animals were from the same herd and therefore had the same risk factors regarding farm management and wildlife exposure. If the follow up period – the number of days between the BCT and the animal's death – had a difference of 90 days or greater between the CITT negative and IC animal, they were not eligible for matching. Animals that were alive at the time of the data extraction were also excluded. All remaining eligible CITT negative animals were included. Therefore, there was a variable number of matched CITT negative animals per IC animal.

IC animals and CITT negative animals were retrospectively followed from the date of the BCT to the date of their death.

2.1.2. Case definition

The outcome of interest was the bTB status of each animal. Animals were considered as bTB positive if:

- they were defined as a reactor at a CITT test which then had gross bTB like lesions at slaughter or were confirmed by histological or bacteriological examination for bTB, or
- were found with bTB-like lesions at routine post mortem examination and subsequently confirmed by histological or bacteriological examination for bTB.

2.1.3. Independent variables

The independent variables included were herd identity, sex, age, production type, herd size and affiliation of the veterinary surgeon (as defined below).

- Sex: Animals were grouped into bulls, females and castrated males.
- Age: For female animals, there were nine age groups ($\leq$ 1 and $>$ 8 years old and yearly age categories in-between). There were three age categories for bulls ($\leq$ 1, 1 to 8 years and $>$ 8 years old) and four age categories for castrated males ($\leq$ 1 year, 1 to 2 years, 2 to 3 years and $>$ 3 years old).
- Production type: Animals were grouped based on breed into dairy and non-dairy. The dairy category included breeds that are mainly used for milk production. The non-dairy category included breeds that are commonly used for beef production.
- Herd size: Herd size was based on the total number of animals tested at the BCT which identified the ICs and categorized using the first and third quartiles, which were 76 and 300 animals respectively. Herds were classified as 'small' ($<$ 76 animals), 'medium' (76 to 300 animals inclusive) or large ($>$ 300 animals).
- Affiliation of veterinary surgeon: Veterinary surgeons were grouped into two categories, government veterinary officers or private veterinary practitioners.

2.1.4. Statistical analysis

The hypothesis tested was that IC animals had a greater risk of becoming bTB positive compared to CITT negative animals after controlling for other confounding factors.

For matched and unmatched ICs that became bTB positive, the number of days between the BCT and being bTB positive was calculated and the herd identity at the time of the BCT and at the time of becoming bTB positive were compared to determine whether they had moved during the time elapsed.

Matched and unmatched ICs at BCTs were compared using the Fisher's exact test for categorical variables (sex, bTB status, Divisional

Veterinary office (DVO), herd type) and Wilcoxon rank sum test for continuous variables (age and herd size). These were used to assess the potential for bias in the selection process.

The proportion of animals that became bTB positive within the IC and CITT negative groups were compared using frequency tables and the McNemar test. The relative risk, risk difference percent and percentage population attributable risk were calculated.

Univariate analysis of the association between each independent variable and the odds of becoming bTB positive was modelled using mixed effects logistic regression. Independent variables were fitted as fixed effects. Each IC animal and their matched CITT negative animals formed cohorts ($n = 204$) of non-independent observations, due to the matching process, so the cohort of matched animals was fitted as a random effect. Independent variables that were associated with becoming bTB positive were examined for potential confounding and interaction.

Two multivariate mixed effect logistic regression models were compared with a univariate model (Model 1) which had being IC at the BCT as a fixed effect and the matched cohort as a random effect. The first multivariate mixed effect logistic regression model (Model 2) included being IC at a BCT, breed and testing veterinary surgeon as fixed effects variables and cohort as a random effect. The second multivariate mixed effect logistic regression model (Model 3) included herd size and being IC at a BCT as fixed effects and cohort as a random effect. Models 2 and 3 were compared to Model 1 using the likelihood ratio test.

For all of the analysis, statistical significance was predetermined at the 5% level ($p < 0.05$).

2.2. Herd level methodology

2.2.1. Eligibility criteria

BCTs within the study period previously defined were eligible for inclusion. If a herd had multiple BCTs within the study period, only the first BCT was included in the analysis. For brevity, herds that had ICs but no CITT reactors at their BCT are referred to as "IC only" herds (exposed cohort). They were compared to herds that had no ICs or CITT reactors and are referred to "clear herds" in this article (unexposed cohort). IC only herds and clear herds were retrospectively followed from the date of their BCT to 26th February 2021.

2.2.2. Case definition

The outcome of interest was the bTB herd status. Herds were considered to have a bTB herd breakdown if they had a CITT reactor with tuberculous-like lesions at post mortem, multiple CITT reactor (independent of lesion status) or laboratory confirmation of *M. bovis* from an animal at routine slaughter.

The number of days between the date of the BCT and the date that they had a bTB herd breakdown was calculated. It is conceivable that, if a herd had a bTB herd breakdown several years after the BCT, that there is no causal relationship between the two events. Therefore, the bTB status over four different time intervals was examined – having a bTB herd breakdown within 365 days of the BCT, between 365 and 730 days, between 730 and 1095 days or at any time after the BCT – based on annual herd testing.

2.2.3. Independent variables

The following independent variables included were:

- Type of Herd: Grouped into two production types – dairy and non-dairy based on having a milk license.
- Size of Herd: This was based on the total number of animals tested at the BCT. The herd size was categorized and classified as small (≤ 50 animals), medium (51 to 100 animals) and large (> 100 animals) based on the median (50 animals) and upper quartile (100 animals) of the distribution.
- Divisional Veterinary Office (DVO): Northern Ireland is divided into ten administrative regions (DVOs). To account for the spatial heterogeneity of bTB herd prevalence, the DVO location of the herd was used as a proxy for local bTB herd prevalence. The DVOs were aggregated into three regions; South East (Armagh, Newry, Newtownards), West (Dungannon, Enniskillen, Strabane, Omagh) and North East (Ballymena, Coleraine, Mallusk) (Fig. 1).
- bTB history: This was defined as the herd having a bTB herd breakdown within the previous year.
- Number of BCT tests: This was categorized as one or multiple BCTs in the 10 year study period.
- Date of BCT: Based on the herd's first BCT in the study period and categorized based on the year.
- Number of ICs at the BCT: This was assessed as a binary variable (no ICs and one or more ICs) and a categorical variable (none, a single IC and multiple ICs). This was the primary independent variable of interest.
- Testing intensity: This was defined as the number of bTB herd tests per year. The numerator was the number of bTB herd tests from the date of the BCT to the time of either the data download for herds that never had a bTB herd breakdown or to the time the herd had their first bTB herd breakdown. The denominator was the number of days between these two dates. The testing intensity was categorized based on quartiles.
- Duration of the breakdown: This was calculated as the number of days from the herd having a bTB herd breakdown to the removal of bTB herd restrictions associated with the breakdown.

2.3. Statistical analysis

The hypothesis tested was that herds that had ICs only at a BCT had a greater odds of a future bTB herd breakdown compared to herds with clear BCTs after controlling for other confounding factors.

The dataset was described using percentages for categorical and binary variables and the median and interquartile range (IQR) for continuous variables. The median time from the BCT to a bTB herd breakdown and the duration of bTB herd breakdown was calculated for IC only herds and clear herds and compared using the Kruskal-Wallis rank sum test.

Univariate analysis of the association between each independent variable and the odds of a bTB breakdown was modelled using mixed effects logistic regression with the year of the BCT as a random effect. Frequency tables, crude odds ratios (OR), 95% confidence intervals and p values were reported. Independent variables were investigated for potential confounding. The region of Northern Ireland was used as a proxy for bTB prevalence as the prevalence varies from 4% in Strabane to 9% in Newtownards. Interaction between the region of Northern Ireland and BCT result was investigated by comparing crude and stratified ORs and using the likelihood ratio test to compare two models; one with and one without an interaction term.

Independent variables that were associated ($p < 0.25$) with herds having a bTB herd breakdown in the univariate analysis were eligible for inclusion. Eligible independent variables were added in a forward stepwise fashion starting with those that had the greatest impact on the OR for the association between the BCT result and subsequent bTB herd breakdown. Independent variables were retained in the model if their p value was below 0.05. Any independent variables that were removed ($p \geq 0.05$), were added again, singularly, at the end to ensure there remained no evidence for an association (Dohoo et al., 2010). The robustness of the multivariate models were assessed using the convergence, the AIC, pseudo-R^2 and the Hosmer-Lemeshow.

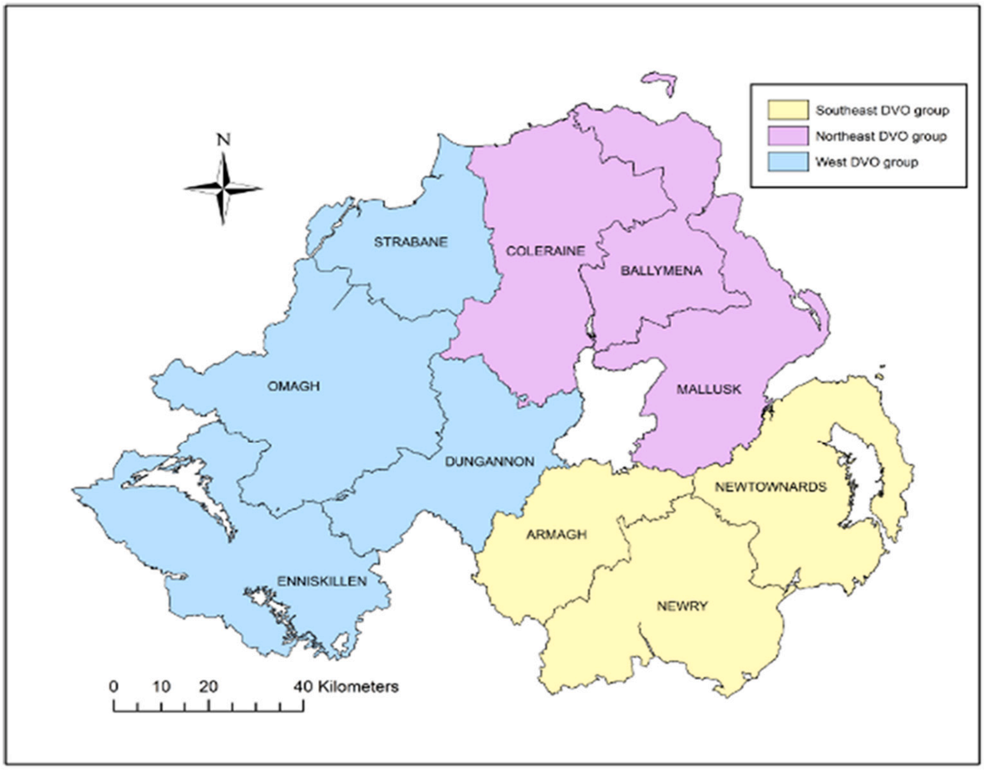

Fig. 1. Divisional Veterinary Offices (DVO) and their regional groupings in Northern Ireland.

3. Results

3.1. Animal level findings

3.1.1. Participants

Initial data extraction identified 360 IC animals and 26,485 CITT negative animals. After applying the matching criteria (sex, age, test ID and follow-up period), the cohorts reduced to 204 IC animals and 914 CITT negative animals. There were 156 IC animals that could not be matched. There were between 1 and 71 CITT negative animals per matched IC animal.

3.1.2. Time until bTB confirmation and movement of ICs

Out of the initial 360 ICs at a BCT, 95 (26.4%) were subsequently found to be positive for bTB. The majority (75%, $n = 71$) were identified at the 42 day IC retest. The time from the BCT to testing positive for bTB ranged from 4 to 2308 days. Most (96%, $n = 91$) became CITT reactors. The rest (4%, $n = 4$) were identified at routine slaughter of an animal. Due to movement restrictions placed on IC animals, most ICs that became TB positive (93%; $n = 88$) were in the same herd throughout the study period. However, of the 24 ICs that were clear at the 42 day retest but subsequently became bTB positive, 29% (n = 7) had moved to an unrestricted herd.

3.1.3. Comparison of matched and unmatched ICs

The matched and unmatched ICs were similar with regards to sex, age at BCT and bTB status (Table 1). However, a greater proportion of matched ICs originated from dairy herds (55.9% versus 26.9%, $p < 0.001$) and, on average, they came from larger herds (median herd size of 149 compared to 77, $p < 0.001$). (See Table 2.)

3.1.4. Descriptive statistics

The median herd size was 149 (interquartile range: 76 to 251). Participant were almost evenly split between beef (51%) and dairy (49%). The median age of participants was 3 years old (IQR: 2 to 5

Table 1
A comparison of matched ($n = 204$) and unmatched ($n = 156$) CITT[a] inconclusive reactors at standard interpretation identified at backward check tests in Northern Ireland between 1st January 2008 and 31st December 2017 (inclusive).

	Matched		Unmatched		P value
	n =	%	n =	%	
Herd Type					
Beef	90	0.441	114	0.731	< 0.001
Dairy	114	0.559	42	0.269	
Sex					
Female	180	0.882	141	0.904	0.609
Male	24	0.118	15	0.096	
Subsequent TB status					
Negative	156	0.765	109	0.699	0.185
Positive	48	0.235	47	0.301	
Area/Region					
South East	81	0.397	41	0.263	0.016
North East	54	0.265	45	0.288	
West	69	0.338	70	0.449	

[a] Comparative intradermal tuberculin test.

Table 2
A comparison of matched (n = 204) and unmatched (n = 156) CITT[a] inconclusive reactors at standard interpretation identified at backward check tests in Northern Ireland between 1st January 2008 and 31st December 2017 (inclusive).

Variable	Min	Median	IQR	Max	P value
Age at BCT (years)					
Matched	1	4	2 to 7	19	0.880
Unmatched	1	5	3 to 7	15	
Herd Size					
Matched	4	149	76 to 251	1195	< 0.001
Unmatched	3	77	42 to 131	694	

[a] Comparative intradermal tuberculin test.

years).

3.1.5. Outcome data

IC animals had 4.5 times the risk (95% CI: 3.03 to 6.34, $p < 0.001$) of becoming bTB positive compared to CITT negative animals. Of the 204 IC animals, 48 were bTB positive. The median number of days from being identified as an IC animal at a BCT and testing bTB positive was 276 days (IQR: 82 to 604 days).

The risk difference percent was 78% meaning that, being an IC at a BCT, was responsible for 78% of subsequent bTB positive animals among animals at a BCT. The percentage population attributable risk was calculated using the probability of exposure and the risk ratio. Given the Rate Ratio of 4.5 and a probability of exposure of 0.00002 (= 360 ICs at BCTs / 16,377,313 animals bTB tested between 1/1/2008 and 31/12/2017), the percentage population attributable risk was 0.002%. Therefore, being an IC at a BCT without reactors, accounts for 0.0001% of all positive bTB animals in Northern Ireland.

3.1.6. Univariate mixed effect logistic regression models

IC animals at a BCT had 2.73 times the odds (95% CI 0.40–6.81, $p < 0.001$) of becoming bTB positive compared to CITT negative animals. There was no evidence that the other independent variables were associated with becoming bTB positive or being an IC at a BCT (Table 3).

3.1.7. Multivariate analysis

There was no evidence that there was interaction between region and BCT results ($p = 0.180$).

The likelihood ratio test provided good evidence ($p = 0.02$) that Model 3, which incorporated being an IC at a BCT and herd size, was a better fit for the data than Model 1, which only incorporated being an IC at a BCT (Table 4). After adjusting for herd size, IC animals at a BCT had 16.16 times the odds (95% CI: 7.75–38.28, $p < 0.001$) of a becoming a bTB positive animal compared to CITT negative animals. The pseudo-R squared was 0.413 and the AIC was 512.4. For Model 1, the pseudo-R^2 was 0.407 and the AIC was 516.2. There was no evidence ($p = 0.459$) that Model 2 (with breed and who completed the BCT included as fixed effects) was a better fit for the data than Model 1. For model 2, the pseudo-R^2 = 0.402 and AIC = 518.7.

Table 3
Summary results from a univariate mixed effect logistic regression for risk factors at a Backward Check Test (BCT) and an individual animal becoming bTB positive, with the matched exposed/unexposed cohort fitted as a random effect, in a retrospective cohort study in Northern Ireland.

Independent Variable	N=	Odds Ratio	95% Confidence Intervals	P value
Exposure Status (Model 1)				
CITT negative	914	-	-	
CITT inconclusive	204	15.04	7.40 to 36.43	< 0.001
Herd Size				
< 75	267	-	-	
76 to 300	582	0.41	0.14 to 1.13	0.088
>300	269	1.31	0.40 to 4.35	0.647
Breed				
Dairy	545	-	-	
Non-dairy	573	0.79	0.35 to 1.71	0.544
Number of BCTs in10 years				
Single	3701	-	-	
Multiple	1010	1.14	0.98 to 1.32	0.088
Type of veterinary surgeon				
Government	1034	-	-	
Private Veterinarian	84	0.44	0.07 to 2.14	0.328
Area/Region				
South East	81	-	-	
North East	54	1.09	0.37 to 3.30	0.863
West	69	3.05	1.18 to 8.49	0.023

Table 4
Multivariable mixed effect logistic regression, with matched exposed/unexposed cohort fitted as a random effect, for the association between CITT inconclusive animals at a Backward Check Test (BCT) and becoming bTB positive in a retrospective cohort study carried out in Northern Ireland.

Model and variables included	N=	Odds Ratio	95% Confidence Intervals	P value
Model 2				
Exposure status				
CITT Negative	914	-	-	
CITT Inconclusive	204	15.27	7.36 to 35.79	<0.001
Breed				
Dairy	545	-	-	
Non-dairy	573	0.69	0.26 to 1.78	0.432
Type of veterinarian				
Government	1034	-	-	
Private Veterinarian	84	0.34	0.04 to 2.35	0.288
Model 3				
Exposure status				
CITT Negative	914	-	-	
CITT Inconclusive	204	16.17	7.75 to 38.28	< 0.001
Herd Size				
< 75	267	-	-	-
76 to 300	582	0.36	0.09 to 1.21	0.105
>300	269	2.02	0.47 to 9.18	0.336

3.2. Herd level findings

3.2.1. Participants

Initial data extraction identified 6207 BCTs from 4711 herds; of which 3701 herds (79%) had a single BCT and 1010 (21%) had multiple BCTs in the 10 year study period. There were 282 IC only BCTs and 4429 clear BCTs. Of the IC only BCTs, 193 (68%) had a single IC and 89 (32%) had multiple ICs at the BCT (Fig. 2).

3.2.2. Descriptive statistics

There were 703 dairy and 4008 non-dairy herds. The median herd size was 47 (IQR: 21 to 101 animals). The median testing intensity was 1.5 herd level CITTs per year (IQR: 1.2 to 1.9).

3.2.3. Outcome data

Approximately 5% of herds ($n = 229$) had recorded a bTB herd breakdown within 365 days prior to their BCT. Less than half of herds ($n = 1970$, 42%) had a bTB herd breakdown after their BCT (Fig. 3).

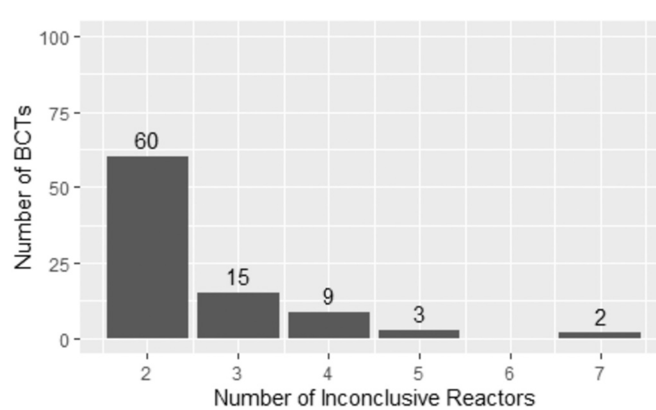

Fig. 2. The number of CITT inconclusive reactors (IC) identified at Backward Check Tests (BCTs), which had no reactors but multiple ICs, in Northern Ireland between 1st January 2008 and 31st December 2017 (inclusive).

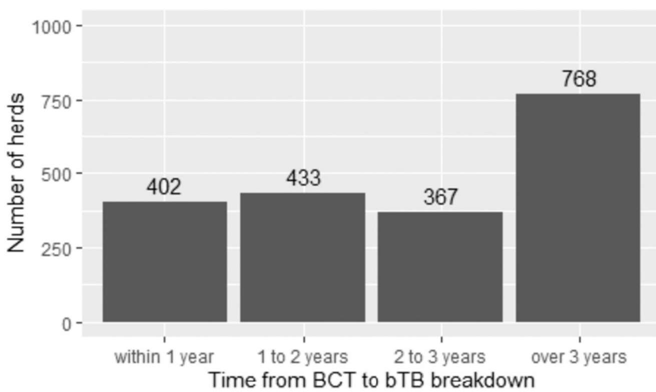

Fig. 3. The number of subsequent bovine tuberculosis (bTB) herd breakdowns, by time interval, after Backward Check Tests (BCT), which had no CITT reactors but multiple inconclusives (IC)s, in Northern Ireland.

3.2.4. Univariate analysis

Herds with ICs only at the BCT had 2.00 times the odds (95% CI: 1.56 to 2.57, $p < 0.001$) of having a bTB herd breakdown compared to herds with a clear test. The evidence for an association between the BCT result and bTB breakdowns was consistent when comparing bTB herd breakdown within 1 year, between 1 and 2 years, between 2 and 3 years and over 3 years after the BCT. The association between other independent variables and the odds of a bTB herd breakdown can be found in Table 5.

3.2.5. Multivariate analysis

There was no evidence ($p = 0.429$) for an interaction between the region and BCT result. After adjusting for confounding by both herd size and type, there was no evidence ($p = 0.201$) that the BCT result was associated with a subsequent bTB herd breakdown. The pseudo-R^2, AIC and Hosmer-Lemeshow p value for each model is shown in Table 6. Balancing model complexity and model fit, the final model contained herd type and herd size as fixed effects and the year of the BCT as a random effect (Table 7). Therefore, after adjusting for confounding, having ICs at a BCT does not appeared to increase the odds of a subsequent bTB herd breakdown compared to herds with a clear BCT.

Table 6
Model performance parameters for mixed effect logistic regression models for the association between having Inconclusive Reactors (ICs) only at a Backward Check Tests (BCT) and the herd subsequently having a bovine tuberculosis (bTB) breakdown, with the year of the BCT as a random effect, in Northern Ireland between 2008 and 2017 (inclusive).

Fixed effect independent variables in the model	AIC	Pseudo R squared	Hosmer-Lemeshow
BCT	6294	0.031	0.983
BCT result Herd Size	5690	0.153	0.920
BCT results Herd size Herd type	5641	0.167	0.651
Herd size Herd type	5641	0.167	0.656
Herd Size Herd type TB history	5621	0.172	0.309
Herd size Herd type TB testing Intensity	5032	0.323	0.023

Table 7
Multivariate mixed effect logistic regression models for factors at a BCT that predict subsequent bovine tuberculosis (bTB) herd breakdowns, with the year of the BCT as a random effect, in Northern Ireland.

	Adjusted OR	95% Confidence Interval	P value
Herd type			
Non-Dairy	–		
Dairy	2.00	1.61 to 2.49	<0.001
Herd size			
< 50	–		
50–100	2.00	1.70 to 2.37	<0.001
> 100	3.79	3.15 to 4.57	<0.001
Testing intensity			
≤ 1.2 tests/yr	–		
1.2 < tests/yr ≤ 1.5	1.86	1.54 to 2.67	<0.001
1.5 < tests/yr ≤ 2	3.10	2.56 to 3.77	<0.001
≥ 2 tests/yr	12.48	9.99 to 15.64	<0.001

Table 5
Univariate mixed effect logistic regression models for risk factors, relating to herd characteristics and findings at Backward Check Tests (BCT), and the bTB herd breakdowns, with the year of the BCT as a random effect, in Northern Ireland.

	N =	Number of bTB herd breakdowns	Crude OR	95% Confidence Interval	P value
BCT results					
Clear	4429	1801	–	–	–
IC only	282	169	2.00	1.56 to 2.57	<0.001
Herd Type					
Non dairy	4008	1468	–		
Dairy	703	502	4.76	3.98 to 5.71	<0.001
Herd Size					
< 50	2455	656	–		–
50–100	1071	493	2.37	2.03 to 2.76	<0.001
> 100	1185	821	6.39	5.48 to 7.47	<0.001
Area					
North East	1059	440	-		
South East	1532	664	1.06	0.90 to 1.25	0.472
West	2120	866	0.97	0.83 to 1.13	0.707
TB breakdown in previous 365 days					
No	4482	1821	–		–
Yes	229	149	2.65	2.00 to 3.52	<0.001
Testing Intensity					
≤ 1.2 tests/yr	1280	270	–	–	–
1.2 < tests/yr ≤ 1.5	1256	429	2.18	1.82 to 2.63	<0.001
1.5 < tests/yr ≤ 2	1217	552	3.78	3.15 to 4.55	<0.001
> 2 test/yr	958	719	16.54	13.36 to 20.56	<0.001

4. Discussion

A Republic of Ireland study found that ICs identified at routine herd testing posed both a short and long term risk of bTB spread with ICs that were slaughtered before their CITT retest being 100 times more likely to have bTB like lesions at post mortem and those that were retested being 1.4 times more likely to be a reactor (Clegg et al., 2011a, 2011b). In the longer term, ICs negative at the CITT retest were 12 times more likely to become a reactor than an animal in the national herd. However, the risk of becoming bTB positive decreased after 500 days. The age of the animal and the bTB herd history were also associated with the risk of an IC subsequently being bTB positive (Clegg et al., 2011a, 2011b). Similarly, in Great Britain, ICs at routine herd testing in England and Wales had a higher odds of becoming reactors compared to negative animals (May et al., 2019). The odds of an IC becoming bTB positive interacted with region level bTB risk with low prevalence areas having a higher odds ratio (low risk area OR = 23; high risk area OR = 6.85). Other factors associated with an IC becoming bTB positive varied between risk areas (May et al., 2019).

This has been replicated in herd level analysis where IC only herds had 2.7 times the hazard of a future TB incident compared to CITT clear herds but with the relative hazard decreasing by 63% annually (Brunton et al., 2018). Other risk factors identified were similar to those controlled for within the current study (herd type, herd size, herd bTB history, regional bTB prevalence).

Our study also found that, after adjusting for confounding factors, ICs at BCTs had a significantly higher risk of becoming bTB positive compared to CITT negative matched animals. However, this study focused on a high risk test (BCTs), which may be more likely to identify bTB positive animals compared to routine herd tests. This may explain the higher odds observed in our study even with matching of cohort animals within the same herd. Although the findings of the individual animal level analysis are consistent with previous research, our study does not replicate the herd level findings of Brunton et al. (2018). There are a number of potential explanations. The current study focused solely on a high risk herd test (BCTs), which may influence test operator behaviour. Also, the current study included tests over a 10 year period compared to a single calendar year.

Given the increased probability of IC animals becoming bTB positive and the fact that a quarter are not removed at the 42 days retest, it may be justifiable to remove ICs from the herd immediately as they are potentially a source of infection for uninfected animals in the herd, neighbouring herds and susceptible wildlife. The median number of day from being identified as an IC and becoming bTB positive is 276, which provides ample time for exposure of uninfected animals and environmental contamination to occur. Over the 10 year study period, there were 360 ICs at BCTs. Therefore, the economic impact of removing ICs at a BCT would be low with roughly 36 additional animals being culled every year. However, 77% of these culls would be removed unnecessarily as they would not subsequently become bTB positive. Whilst being an IC at a BCT has a large observed effect for individual animals, at the population level, the impact is small. Assuming causality, ICs at a BCT without reactors are responsible for 0.002% of all bTB positive animals in Northern Ireland. In addition, given that the time lapse between the BCT and being identified as bTB positive can be up to 2308 days, it is possible that some of these animals were exposed to and infected with bTB after being identified as an IC. This means that the true percentage population attributable risk is likely to be lower than 0.0001%. Therefore, removal of ICs at a BCT without reactors may only have a small impact on the overall bTB statistics for Northern Ireland although the cumulative effect over time also has to be considered. However, given that almost a third (29%) of ICs that are negative at the 42 day retest but subsequently become bTB positive, move to an unrestricted herd, it may be justifiable to place lifetime movement restrictions to the herd of disclosure on ICs identified at BCTs.

These findings have the potential to impact bTB policy in Northern Ireland. A successful eradication programme must address the multiple and diverse mechanisms of bTB transmission including recurrence and recrudescence. Residual infection in cattle is increasingly acknowledged as an important feature of bovine infections with *M. bovis* in the UK (Conlan et al., 2015) and is the most likely explanation for the increased bTB risk in ICs, found in this and other studies.

In the Republic of Ireland, since 2012, ICs that are CITT negative when re-tested are restricted to the herd in which they were detected for life. Introducing a similar policy in Northern Ireland may provide a balance between the increased risk posed by ICs whilst acknowledging that the majority will not become bTB positive.

The CITT has a moderate sensitivity. Therefore, the presence of ICs at a BCT may indicate that there are further bTB positive animals in the herd that have been misclassified as negative that will be unveiled at future tests. However, given there was no evidence that herds with IC only animals at a BCT had a significantly increased odds of a future bTB breakdown once confounding factors were accounted for, this is unlikely to be the case. Therefore, taking more stringent action at the herd level seems unwarranted.

ICs that were matched and included in the retrospective cohort study were more likely to be from large herds and dairy herds. Previous studies have found that larger herds and dairy herds are more likley to have bTB breakdowns (Skuce et al., 2012; Doyle et al., 2016; Wright et al., 2015). Therefore, this has the potential to introduce bias into the current study. However, the proportion of ICs that became confirmed bTB reactors was similar in matched and unmatched cohorts suggesting that the impact of herd size and type was minimal.

This study utilised data collected as part of the bTB control and eradiation programme in Northern Ireland. The APHIS database contains a vast amount of useful and accurate data. Nevertheless, factors that previous studies have shown to be influential, such as wildlife density and interaction with domestic livestock, may not have been recorded and hence not controlled for within these analyses. Previous studies have identifed animal movements as a risk factor (Broughan et al., 2016; Doyle et al., 2016). This study did not include animal movements because, whilst herd size and type are not a good proxy for the number of animal movements in GB, the farming industry in Northern Ireland is distinctively different (Abernethy et al., 2006), with larger herds, in general, having more movements and dairy herds being less dependant than beef on live animal sales. In addition, BCT's by their very nature are carried out in herds which have sold animals on to other herds. The herds most at risk of bTB due to high turnover are large beef fattener/finisher herds. However, these herds move all their finished cattle to abattoirs and therefore do not initiate BCTs. The complex nature of bTB epidemiology alongside time lags, especially between testing intervals and data exclusion, may explain the low pseudo-R squared with 40% of the variable in future bTB status of animals and 17% of the variability in subsequent bTB breakdowns being accounted for.

5. Conclusions

There is a strong indication that ICs at a BCT pose a higher risk of bTB infection compared to clear animals at the same BCT; although this is not reflected at the herd level. Therefore, it is recommended that all ICs at BCTs are either removed from the herd or have lifetime movement restrictions placed upon such individuals.

Declaration of Competing Interest

None.

References

Abernethy, D.A., Denny, G.O., Menzies, F.D., McGuckian, P., Honhold, N., Roberts, A.R., 2006. The Northern Ireland programme for the control and eradication of Mycobacterium bovis. Vet. Microbiol. 112, 231–237. https://doi.org/10.1016/j.vetmic.2005.11.023.

Barton, K., 2020. MuMIn: Multi-Model Inference. R package version 1 (43), 17. https://CRAN.R-project.org/package=MuMIn.

Bates, D., Maechler, M., Bolker, B., Walker, S., 2015. Fitting linear mixed-effects models using lme4. J. Stat. Softw. 67, 1–48. https://doi.org/10.18637/jss.v067.i01.

Broughan, J.M., Judge, J., Ely, E., Delahay, R.J., Wilson, G., Clifton-Hadley, R.S., Goodchild, A.V., Bishop, H., Parry, J.E., Down, S.H., 2016. A review of risk factors for bovine tuberculosis infection in cattle in the UK and Ireland. Epidemiol. Infect. 144, 2899–2926.

Brunton, L.A., Prosser, A., Pfeiffer, D.U., Downs, S.H., 2018. Exploring the fate of cattle herds with inconclusive reactors to the tuberculin skin test. Front. Vet. Sci. 28, 5–228.

Campbell, E.L., Menzies, F.D., Byrne, A.W., Porter, S., McCormick, C.M., McBride, K.R., Scantlebury, M.D., Reid, N., 2020. Grazing cattle exposure to neighbouring herds and badgers in relation to bovine tuberculosis risk. Res. Vet. Sci. 133, 297–303.

Clegg, T.A., Good, M., Duignan, A., Doyle, R., Blake, M., More, S.J., 2011a. Longer-term risk of Mycobacterium bovis in Irish cattle following an inconclusive diagnosis to the single intradermal comparative tuberculin test. Prev.Vet. Med. 100, 3–4.

Clegg, T.A., Good, M., Duignan, A., Doyle, R., More, S.J., 2011b. Shorter-term risk of *Mycobacterium bovis* in Irish cattle following an inconclusive diagnosis to the single intradermal comparative tuberculin test. Prev. Vet. Med. 102, 255–264.

Conlan, A.J., Brooks Pollock, E., McKinley, T.J., Mitchell, A.P., Jones, G.J., Vordermeier, M., Wood, J.L., 2015. Potential benefits of cattle vaccination as a supplementary control for bovine tuberculosis. PLoS Comput. Biol. 11 (2), e1004038.

DAERA, 2018. TB Staff Instructions (DAERA internal document).

DAERA, 2021. Tuberculosis - disease statistics - January 2021. Retrieved from: www.daera-ni.gov.uk/articles/tuberculosis-statistics-northern-ireland. Accessed 30 September 2021.

Dohoo, I., Martin, W., Stryhn, H., 2010. Model building strategies. In: Veterinary Epidemiologic Research. VER Inc., Charlottetown, pp. p383–p388.

Doyle, L.P., Courcier, E.A., Gordon, A.W., O'Hagan, M.J.H., Menzies, F.D., 2016. Bovine tuberculosis in Northern Ireland: risk factors associated with duration and recurrence of chronic herd breakdowns. Prev. Vet. Med. 131, 1–7.

Godfray, H.C., Donnelly, C.A., Kao, R.R., Macdonald, D.W., McDonald, R.A., Petrokofsky, G., Wood, J.L.N., Woodroffe, R., Young, D.B., McLean, A.R., 2013. A restatement of the natural science evidence base relevant to the control of bovine tuberculosis in Great Britain. Proc. Biol. Sci. 280, 20131634.

Houston, R., 2001. A computerised database system for bovine traceability. Rev. Sci. Tech. 20, 652–661.

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

May, E., Prosser, A., Downs, S.H., Brunton, L.A., 2019. Exploring the risk posed by animals with an inconclusive reaction to the bovine tuberculosis skin test in England and Wales. Vet Sci. 30, 97.

More, S.J., 2009. What is needed to eradicate bovine tuberculosis successfully: an Ireland perspective. Vet. J. 180, 275–278.

More, S.J., Good, M., 2015. Understanding and managing bTB risk: perspectives from Ireland. Vet. Microbiol. 176, 209–218.

Northern Ireland Audit Office, 2018. Eradicating Bovine TB in Northern Ireland. Retrieved from. www.niauditoffice.gov.uk/sites/niao/files/media-files/NIAO_%20Bovine%20Tuberculosis%20report.pdf. Accessed 30 September 2021.

O'Hagan, M.J.H., Ni, H., Menzies, F.D., Pascual-Linaza, A.V., Georgaki, A., Stegeman, J. A., 2019. Test characteristics of the tuberculin skin test and post-mortem examination for bovine tuberculosis diagnosis in cattle in Northern Ireland estimated by Bayesian latent class analysis with adjustments for covariates. Epidemiol. Infect. 147, e209.

Orton, R.J., Deason, M., Bessell, P.R., Green, D.M., Kao, R.R., Salvador, L.C.M., 2018. Identifying genotype specific elevated-risk areas and associated herd risk factors for bovine tuberculosis spread in British cattle. Epidemics. 24, 34–42.

Sedighi, T., Varga, L., 2021. Evaluating the bovine tuberculosis eradication mechanism and its risk factors in England's cattle farms. Int. J. Environ. Res. Public Health 18, 3451.

Sheridan, M., 2011. Progress in tuberculosis eradication in Ireland. Vet. Microbiol. 151, 160–169.

Skuce, R.A., Allen, A.R., McDowell, S.W., 2011. Bovine Tuberculosis (TB): A Review Of Cattle-To-Cattle Transmission, Risk Factors And Susceptibility. Retrieved from. www.daera-ni.gov.uk/sites/default/files/publications/dard/afbi-literature-review-tb-review-cattle-to-cattle-transmission.pdf. Accessed 13th October 2021.

Skuce, R.A., Allen, A.R., McDowell, S.W., 2012. Herd-level risk factors for bovine tuberculosis: a literature review. Vet. Med. Int. 621210.

Wickham, H., Averick, M., Bryan, J., Chnag, W., McGowan, L., Francois, R., Grolemund, G., Hayes, A., Henry, L., Hester, J., Kuhn, M., Pedersen, T.L., Miller, E., Bache, S.M., Muller, K., Ooms, J., Robinson, D., Seidel, D.P., Spinu, V., Takahashi, K., Vaughan, D., Wilke, C., Woo, K., Yutani, H., 2019. Welcome to the tidyverse. J. Open Source Softw. 4, 1686. https://doi.org/10.21105/joss.01686.

World Organisation of Animal Health, 2018. Chapter 3.4.6 – Bovine tuberculosis. In: OIE Terrestrial manual, pp. 1058–1074.

Wright, D.M., Reid, N., Ian Montgomery, W., Allen, A.R., Skuce, R.A., Kao, R.R., 2015. Herd-level bovine tuberculosis risk factors: assessing the role of low-level badger population disturbance. Sci. Rep. 5, 13062.

Zeileis, A., Hothorn, T., 2002. Diagnostic checking in regression relationships. R News 2, 7–10. https://CRAN.R-project.org/doc/Rnews/.