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Original Paper

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Abstract

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

Introduction

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

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

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

Animals infected with environmental mycobacteria can also react positively to the SCITT, but there will normally be no evidence of bTB related visible lesions. However, the specificity of the SCITT has been estimated at over 99.9%, indicating that a SCITT false positive result is a rare event [8, 9]. The sensitivity of the SCITT shows great variation; a median of 80% (range 75–96%) at standard interpretation, highlighting the risk of residual infection in herds has been reported [8]. Bayesian analytical techniques have suggested even lower SCITT sensitivity levels (50%) [10].

Currently policies relating to requirements for cattle herds to regain Official Tuberculosis Free (OTF) status after a bTB incident vary across the British Isles from only requiring one negative herd-level SCITT if infection is not confirmed (termed OTS regimen; as outlined in European Directive 64/432/EEC, as amended), to requiring two consecutively negative herd-level SCITTs (termed OTW regimen) after disclosure of two or more SCITT reactors, even if no confirmation of infection is found. In Northern Ireland, at the time of writing herds with five or less unconfirmed SCITT reactors need only one clear herd-level SCITT, whereas all other incidents require two clear consecutive herd-level SCITTs at intervals of 42–60 days in order to regain OTF status.

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

Materials and methods

Study design and study population

An observational, retrospective cohort study was conducted with the study population including all new bTB herd incidents occurring during 2008 that had a 6-month follow-up herd-level SCITT Check Herd Test (CHT) after withdrawal of movement restrictions following the bTB incident. Herds that had an initial herd-level SCITT follow-up that was not a CHT were excluded from the analysis as they may have been at increased risk from other factors such as being contiguous to another bTB herd incident. New bTB herd incidents that were initiated by bTB detection in an animal at routine slaughter without any subsequent SCITT reactors were also excluded. A new bTB herd incident for the sampling frame was defined as a herd that had one or more SCITT reactors in 2008 with no SCITT reactors during the previous 12 months. Study herds were followed up for a 4-year period after regaining OTF status following the end of the initial bTB herd incident (Fig. 1). Within that 4-year follow-up period, a herd was considered to be a bTB incident again if SCITT reactors or bTB detection in animals at routine slaughter were disclosed. The methodology used for the survival study was broadly based on a study design previously used in the Republic of Ireland [12].

Data collection and definition of variables

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

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

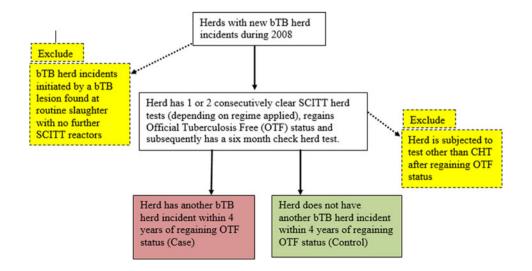


Fig. 1. Diagram outlining the study design.

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Number of SCITT reactors during the initial bTB herd incident	Number of Number of herds wi herds future breakdow		Median time in days to next incident for herds with an incident during the follow-up period	Absolute risk for future breakdown (%)	
1	507	160	902	31.6	
2	182	76	803	41.8	
3	91	39	592	42.9	
4	59	26	546	44.1	
5	38	19	586	50.0	
>5	159	88	534	55.3	
Total	1036	408	708	39.4	

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

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

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

Data analyses

Survival analyses-based plots of the Kaplan-Meier estimators were conducted to evaluate the survival rate of study herds [20], focused on the number of SCITT reactors during the bTB herd incident and the bTB confirmation status of the incident. Further survival analyses were conducted on a subset of data

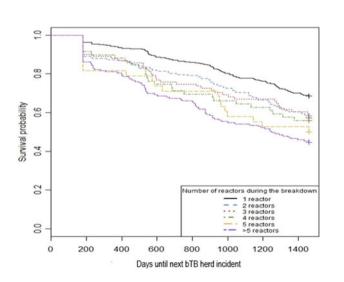


Fig. 2. Kaplan–Meier curves by a number of SCITT reactors during the initial bTB herd incident.

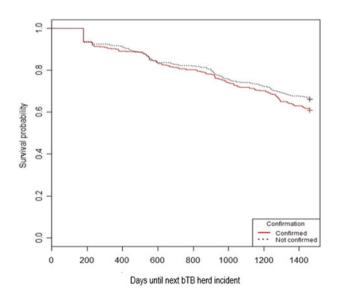


Fig. 3. Kaplan-Meier curves by bTB confirmation status during the initial bTB herd incident.

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

		Future bTB incident (N = 408)		re bTB incident (N = 628)				
Variable	п	% cases	N	% controls	Hazard ratio	95% CI	P value (Wald tes	
Number of SCITT reacto	ors during incide	ent						
1	160	39.2	347	55.3	1.000	-	<0.001	
2	76	18.6	106	16.9	1.468	1.118-1.930		
3	39	9.6	52	8.3	1.553	1.094-2.203		
4	26	6.4	33	5.3	1.575	1.040-2.384		
5	19	4.7	19	3.0	1.893	1.177-3.046		
>5	88	21.6	71	11.3	2.300	1.772-2.984		
Number of SCITT reacto	ors at disclosure	test						
1	195	47.8	377	60.0	1.000	-	<0.001	
2	71	11.5	96	15.3	1.350	1.029-1.772		
3	28	6.9	49	7.8	1.114	0.750-1.655		
4	27	6.6	31	4.9	1.558	1.042-2.330		
5	18	4.4	16	2.5	1.733	1.069-2.808		
>5	69	16.9	59	9.4	1.965	1.493-2.587		
Herd size								
Per animal increase					1.003	1.002-1.003	<0.001	
bTB confirmation								
No	136	33.3	212	33.8	1.000	-	0.821	
Yes	272	66.7	416	66.25	1.024	0.834-1.258		
Divisional Veterinary Of	fice area							
Armagh	31	7.6	50	8.0	1.000	-	0.104	
Ballymena	19	4.7	37	5.9	0.861	0.486-1.524		
Coleraine	49	12.0	76	12.1	1.038	0.662-1.627		
Dungannon	47	11.5	75	11.9	0.998	0.634-1.570		
Enniskillen	57	14.0	113	18.0	0.831	0.537-1.287		
Londonderry	15	3.7	33	5.3	0.825	0.466-1.460		
Mallusk	19	4.7	35	5.6	0.758	0.409-1.403		
Newry	80	19.6	87	13.9	1.415	0.934-2.142		
Newtownards	45	11.0	46	7.3	1.482	0.938-2.342		
Omagh	46	11.3	76	12.1	0.946	0.600-1.492		
bTB in previous 3 years	i							
No	251	61.5	455	72.5	1.000	-	<0.001	
Yes	157	38.5	173	27.5	1.513	1.239-1.847		
Herd type								
Non-dairy	233	57.1	479	76.3	1.000	-	<0.001	
Dairy	175	42.9	149	23.7	1.936	1.591-2.356		
Number of animals mov	ved into the her	d 90 days before	the bTB herc	l incident or the end	d of the follow-up peri	od		
0	38	9.3	101	16.1	1.000	-	<0.001	
1–5	58	14.2	174	27.7	0.850	0.564-1.279		
6–13	91	22.3	160	25.5	1.322	0.905-1.930		
14-33	123	30.1	127	20.2	2.106	1.463-3.030		
>33	98	24.0	66	10.5	2.895	1.990-4.211		

Table 2. (Continued.)

		Future bTB incident (N = 408)		re bTB incident (N = 628)			
Variable	п	% cases	Ν	% controls	Hazard ratio	95% CI	P value (Wald test)
Patch bTB prevalence							
<q1< td=""><td>89</td><td>21.8</td><td>170</td><td>27.1</td><td>1.000</td><td>-</td><td><0.001</td></q1<>	89	21.8	170	27.1	1.000	-	<0.001
≥Q1 to <med< td=""><td>91</td><td>22.3</td><td>166</td><td>26.4</td><td>1.071</td><td>0.800-1.434</td><td></td></med<>	91	22.3	166	26.4	1.071	0.800-1.434	
≥Med to <q3< td=""><td>95</td><td>23.3</td><td>163</td><td>26.0</td><td>1.112</td><td>0.833-1.485</td><td></td></q3<>	95	23.3	163	26.0	1.112	0.833-1.485	
≽Q3	133	32.6	129	20.5	1.871	1.430-2.447	

based on bTB herd incidents that had no further SCITT reactors after the disclosure test (categorised by a number of reactors).

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

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

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

Results

Descriptive results

There were 1036 new bTB herd incidents during 2008 that had a six-month follow-up herd-level SCITT CHT after derestriction from the bTB herd incident. Of those herds, 408 (39.4%) had a future bTB herd incident within the follow-up time.

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

Survival analyses

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

Cox regression model

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

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

The final multivariable model (Table 3) consisted of the number of SCITT reactors during the bTB herd incident, herd size, bTB history in the previous 3 years, DVO area, herd type, purchase intensity and patch bTB prevalence. Increasing number of SCITT reactors during the bTB herd incident was associated with a significantly increased risk of a future bTB herd incident (hazard ratio = 1.861 in incidents >5 SCITT reactors compared to incidents with only one SCITT reactor; 95% CI 1.412–2.453; P < 0.001). Increasing herd size was also significantly associated with the risk of future bTB herd incident (hazard ratio = 1.002 per animal increase; 95% CI 1.001–1.003; P < 0.001). As were herd type (hazard ratio dairy herds *vs.* non-dairy herds = 1.271; 95%

Variable	Future bTB incident (N = 408)		No future bTB incident (N = 628)					
	п	% cases	п	% controls	Hazard ratio	95% CI	P value	P value
Number of SCITT reactors	during incide	ent						
1	160	39.2	347	55.3	1.000	-	-	<0.001
2	76	18.6	106	16.9	1.380	1.045-1.821	0.023	
3	39	9.6	52	8.3	1.579	1.104-2.257	0.012	
4	26	6.4	33	5.3	1.461	0.956-2.233	0.080	
5	19	4.7	19	3.0	1.585	0.975-2.578	0.063	
>5	88	21.6	71	11.3	1.861	1.412-2.453	<0.001	
Herd size								
Per animal increase					1.002	1.001-1.003	<0.001	<0.001
bTB in previous 3 years								
No	251	61.5	455	72.5	1.000	-		0.313
Yes	157	38.5	173	27.5	1.152	0.931-1.425	0.193	
Divisional veterinary office	area							
Armagh	31	7.6	50	8.0	1.000	-	-	0.109
Ballymena	19	4.7	37	5.9	0.788	0.438-1.418	0.426	
Coleraine	49	12.0	76	12.1	1.101	0.690-1.756	0.686	
Dungannon	47	11.5	75	11.9	1.361	0.851-2.177	0.198	
Enniskillen	57	14.0	113	18.0	0.754	0.477-1.192	0.227	
Londonderry	15	3.7	33	5.3	0.799	0.443-1.440	0.456	
Mallusk	19	4.7	35	5.6	0.630	0.337-1.178	0.148	
Newry	80	19.6	87	13.9	1.444	0.936-2.228	0.097	
Newtownards	45	11.0	46	7.3	1.137	0.711-1.820	0.591	
Omagh	46	11.3	76	12.1	1.323	0.823-2.124	0.248	
Herd type								
Non-dairy	233	57.1	479	76.3	1.000	-	-	0.021
Dairy	175	42.9	149	23.7	1.271	1.018-1.587	0.035	
Number of animals moved	into the her	rd 90 days befor	e the incider	nt in follow-up perio	d or end of follow-u	p period		
0	38	9.3	101	16.1	1.000	_	-	<0.001
1–5	58	14.2	174	27.7	0.775	0.513-1.171	0.226	
6–13	91	22.3	160	25.5	1.096	0.743-1.615	0.645	
14-33	123	30.1	127	20.2	1.402	0.953-2.063	0.087	
>33	98	24.0	66	10.5	1.646	1.067-2.539	0.024	
Patch bTB prevalence								
<q1< td=""><td>89</td><td>21.8</td><td>170</td><td>27.1</td><td>1.000</td><td>_</td><td>-</td><td><0.001</td></q1<>	89	21.8	170	27.1	1.000	_	-	<0.001
≥Q1 to <med< td=""><td>91</td><td>22.3</td><td>166</td><td>26.4</td><td>0.926</td><td>0.675-1.269</td><td>0.631</td><td></td></med<>	91	22.3	166	26.4	0.926	0.675-1.269	0.631	
≥Med to <q3< td=""><td>95</td><td>23.3</td><td>166</td><td>26.0</td><td>0.910</td><td>0.669–1.238</td><td>0.549</td><td></td></q3<>	95	23.3	166	26.0	0.910	0.669–1.238	0.549	
≥Q3	133	32.6	126	20.5	1.761	1.298-2.392	<0.001	

CI 1.018–1.587; P = 0.035), purchase intensity (hazard ratio = 1.646 (95% CI 1.067–2.539; P = 0.024) if >33 animals moved into the herd 90 days before the incident in the follow-up period or end of follow-up period) and patch bTB prevalence (hazard ratio =

1.761; 95% CI 1.298–2.392; P < 0.001 for upper quartile compared with lowest quartile). Schoenfeld residuals of the multivariable model showed that the proportional hazard assumption was not violated.

Discussion

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

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

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

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

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

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

Conclusion

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

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References

- Menzies FD and Neill SD (2000) Cattle-to-cattle transmission of bovine tuberculosis. Veterinary Journal 160, 92–106.
- Pollock JM et al. (2006) Pathogenesis of bovine tuberculosis: the role of experimental models of infection. Veterinary Microbiology 112, 141–150.
- Fenhalls G et al. (2000) In situ production of interferon-γ, interleukin-4, and tumour necrosis factor α mRNA in human lung tuberculous granulomas. Infection and Immunity 68, 2827–2836.
- Abernethy DA et al. (2006) The Northern Ireland programme for the control and eradication of *Mycobacterium bovis*. Veterinary Microbiology 112, 231–237.

- O'Hagan MJH et al. (2015) Risk factors for visible lesions or positive laboratory tests in bovine tuberculosis reactor cattle in Northern Ireland. Preventive Veterinary Medicine 120, 283–290.
- Corner LA (1994) Post mortem diagnosis of Mycobacterium bovis infection in cattle. Veterinary Microbiology 40, 53–63.
- Whipple DL, Bolin CA and Miller JM. (1996) Distribution of lesions in cattle infected with Mycobacterium bovis. Journal of Veterinary Diagnostic Investigation 8, 351–354.
- De la Rua-Domenech R et al. (2006) Ante-mortem diagnosis of tuberculosis in cattle: a review of the tuberculin tests, gamma-interferon assay and other ancillary diagnostic techniques. *Research in Veterinary Science* 81, 190–210.
- Goodchild AV et al. (2015) Specificity of the comparative skin test for bovine tuberculosis in Great Britain. Veterinary Record 177(258). doi: 10.1136/vr.102961.
- Nuñez-Garcia J et al. (2017) Meta-analyses of the sensitivity and specificity of ante-mortem and post-mortem diagnostic tests for bovine tuberculosis in the UK and Ireland. *Preventive Veterinary Medicine* 153, 94-107.
- Karolemeas K et al. (2010) Predicting prolonged bovine tuberculosis incidents in Great Britain as an aid to control. *Preventive Veterinary Medicine* 97, 183–190.
- 12. Olea-Popelka FJ et al. (2004) Breakdown severity during a bovine tuberculosis episode as a predictor of future herd incidents in Ireland. *Preventive Veterinary Medicine* 63, 163–172.
- Wolfe DM et al. (2010) From explanation to prediction: a model for recurrent bovine tuberculosis in Irish cattle herds. *Preventive Veterinary Medicine* 94, 170–177.
- 14. Murray D, Clegg TA and More SJ (2012) Evaluation of single reactor bovine tuberculosis incidents based on analysis of reactors slaughtered at an Irish Export meat plant. *Veterinary Record* **170**, 516–521.
- Doyle LP et al. (2014) Bovine tuberculosis in Northern Ireland: risk factors associated with time from post-outbreak test to subsequent herd incident. Preventive Veterinary Medicine 131, 1–7.
- Clegg TA, Good M and More SJ (2015) Future risk of bovine tuberculosis recurrence among higher risk herds in Ireland. *Preventive Veterinary Medicine* 118, 71–79.
- Houston R (2001) A computerised database system for bovine traceability. Revenue Scientific et Technique International Office of Epizootics 20, 652–661.
- Therneau T. A Package for Survival Analysis in S. version 2.38. Available at https://CRAN.R-project.org/package=survival.
- Doyle LP et al. (2016) Bovine tuberculosis in Northern Ireland: risk factors associated with duration and recurrence of chronic herd breakdowns. Preventive Veterinary Medicine 131, 1–7.
- Kaplan EL and Meier P. (1958) Non parametric estimation from incomplete observations. *Journal of the American Statistical Association* 53, 457–481.
- 21. Cox DR and Oakes D. (1984) Analysis of Survival Data. London: Chapman and Hall. pp. 91–111.

- 22. Katz MH (2006) Multivariable Analysis, A Practical Guide for Clinicians. New York: Cambridge University Press, pp. 43–47; 137–152.
- 23. Bradburn MJ et al. (2003) Survival analysis part III: multivariate data analysis choosing a model and assessing its adequacy and fit. British Journal of Cancer 18, 605–611.
- 24. Grambsch PM and Therneau TM (1994) Proportional hazards tests and diagnostics based on weighted residuals. *Biometrika* **81**, 515–526.
- Peduzzi P et al. (1995) Importance of events per independent variable in proportional hazards regression analyses II. Accuracy and precision of regression estimates. *Journal of Clinical Epidemiology* 48, 1503–1510.
- Gallagher MJ et al. (2013) Comparison of bovine tuberculosis recurrence in Irish herds between 1998 and 2008. Preventive Veterinary Medicine 111, 237–244.
- 27. EFSA (2012) Scientific Opinion on the use of a gamma interferon test for the diagnosis of bovine tuberculosis. EFSA Journal 10, 2975.
- Clegg TA et al. (2011) Using latent class analysis to estimate the test characteristics of the gamma-interferon test, the single intradermal comparative tuberculin test and a multiplex immunoassay under Irish conditions. *Veterinary Microbiology* 151, 68–76.
- 29. Skuce RA, Allen AR and McDowell SWJ (2012) Herd-level risk factors for bovine tuberculosis: a literature review. *Veterinary Medicine International* 2012, 1–10. doi: 10.1155/2012/621210.
- Broughan JM et al. (2016) A review of risk factors for bovine tuberculosis infection in cattle in the UK and Ireland. *Epidemiology and Infection* 144, 2899–2926.
- 31. **TB HUB**. Available at http://www.tbhub.co.uk/tb-policy/england/ (Accessed 21 November 2017).
- DAFM. Available at https://www.agriculture.gov.ie/media/migration/ animalhealthwelfare/diseasecontrols/tuberculosistbandbrucellosis/diseaseeradicationpolicy/VeterinaryHandbook2017150217.pdf (Accessed 21 November 2017).
- Good M et al. (2011) Impact of the national full herd depopulation policy on the recurrence of bovine tuberculosis in Irish herds, 2003–2005. *Veterinary Record* 169, 581–585.
- Frankena K et al. (2007) Quantification of the relative efficiency of factory surveillance in the disclosure of tuberculosis lesions in attested Irish cattle. *Veterinary Record* 161, 679–684.
- 35. Olea-Popelka F et al. (2012) Relative effectiveness of Irish factories in the surveillance of slaughtered cattle for visible lesions of tuberculosis 2005–2007. Irish Veterinary Journal 65, 2. doi: 10.1186/2046-0481-65-2.
- Wright DM et al. (2013) Detectability of bovine TB using the tuberculin skin test does not vary significantly according to pathogen genotype within Northern Ireland. *Infection, Genetics and Evolution* 19, 15–22.
- Pascual-Linaza AV et al. (2016) Efficiency of slaughter house surveillance for the detection of bovine tuberculosis in cattle in Northern Ireland. Epidemiology and Infection 145, 995–1005.
- Pollock JM, Welsh MD and McNair J (2005) Immune responses in bovine tuberculosis: towards new strategies for the diagnosis and control of disease. *Veterinary Immunology and Immunopathology* 108, 37–43.