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

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Abstract

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

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

These risk factors are likely related to the time since infection, the strength of the challenge of infection and the susceptibility of the animal. These findings are important as the detection of visible lesions and the confirmation of bTB are an integral part of the overall bTB control programme in Northern Ireland and the veterinary meat inspection

and hygiene programme. The visible lesion status and bTB status of an animal can affect the way in which bTB breakdowns are managed, since failure to detect visible lesions and recovery of *M. bovis* can lead to a less stringent follow-up after other risk factors have been taken into account.

1. Introduction

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

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

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

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

In developed countries, the main purpose of meat inspection in relation to bTB is to act as an ancillary surveillance system and it is an essential component of the overall control

programme (Olea-Popelka *et al*., 2008). The sensitivity of gross post-mortem examination depends on the method employed and the anatomical sites examined. The detection rate of visible lesions varies significantly between abattoirs (Frankena *et al.*, 2007; Olea-Popelka *et al.*, 2012; Shittu *et al.*, 2013; Wright *et al.*, 2013). In Northern Ireland all reactors with visible lesions are subjected to histology examination of which the majority shows tuberculoid granulomata. These samples are subsequently reported as having a positive bTB status. Those samples that do not demonstrate tuberculoid granulomata on histological examination are subjected to bacterial culture. Lymph node tissue samples from reactors without visible lesions are trimmed, serial sliced and examined for lesions. If no lesions are found these samples are subjected to bacteriological culture only. The likelihood of culturing *M. bovis* is greatly increased by sampling from macroscopic lesions ("Visible lesions" or "VL") and/or by thinly slicing lungs of infected cattle (DEFRA, 2007; OIE, 2009). Lack of macroscopic lesions ("Non visible lesions" or NVL) could be due to early infection, the poor sensitivity of the post-mortem examination or infection with Mycobacteria other than *M. bovis* (Corner, 1994). The purpose of this study was to determine the risk factors associated with the presence of visible bTB lesions or positive laboratory tests (i.e. histology and culture) in cattle that reacted positively to the SICCT with the aim of being able to use this information to inform and improve disease control.

2. Materials and methods

2.1. Study population

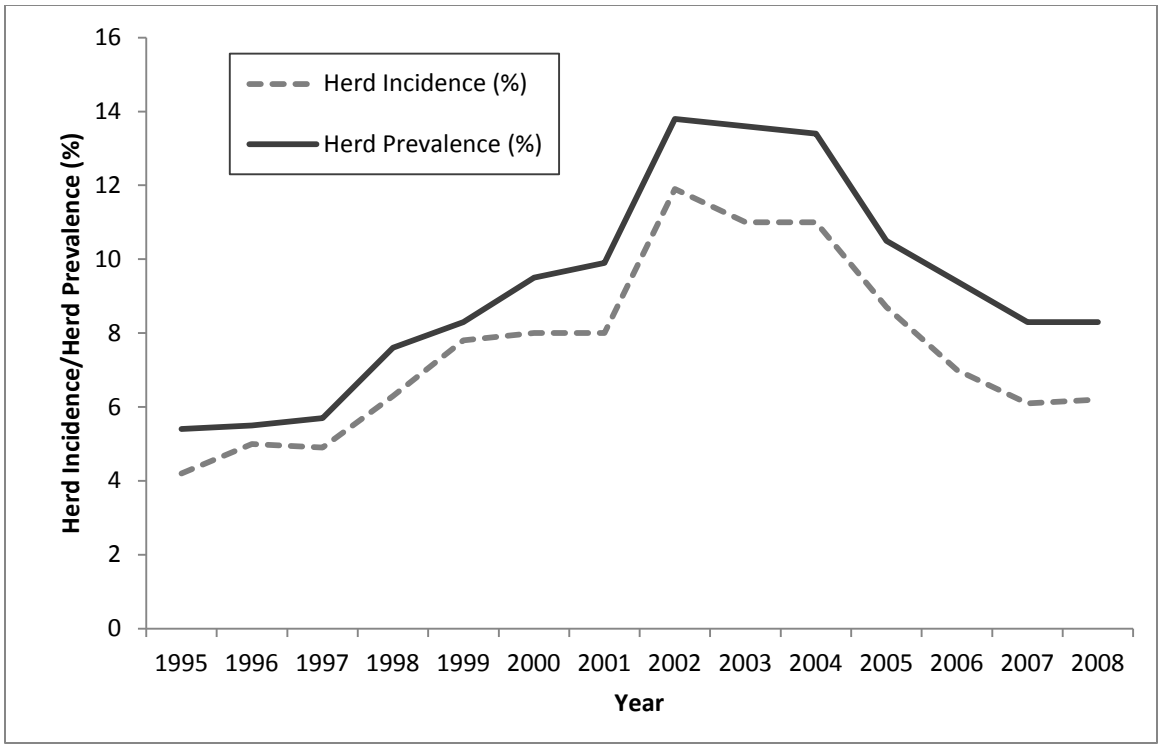


Figure 1: Bovine tuberculosis herd incidence and herd prevalence in Northern Ireland between1995 and 2008.

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

2.2. Data source

Data were extracted from the central animal health database (APHIS) of the Department of Agriculture and Rural Development for Northern Ireland (DARD), which contains the details of all individual cattle, cattle holdings, cattle movements and cattle tuberculosis and brucellosis tests in Northern Ireland since 1988 (Houston, 2001). Complete information was available on the animal health data base for reactor animals in relation to their place of slaughter for the years 2002 and 2006. In 2002, 99.9% of reactor animals were slaughtered in one of three abattoirs. These reactors were nearly evenly distributed over these three abattoirs (respectively 27.7%, 33.0% and 39.2%). However, in 2006 one of these three abattoirs became the destination for 91.7% of reactor animals. Post mortem inspection for evidence of bTB lesions in reactor animals includes the examination of the lymphnodes of the head, chest and mesenterium, lungs, pleura, peritoneum, prescapular lymphnodes, popliteal lymphnodes, iliac lymphnodes and the precrural lymph nodes. Not all reactor animals are subjected to laboratory testing depending on the number of reactors in the herd and lesion status of the animals. For every breakdown samples for up to 5 reactors without VL and 3 VL reactors are subjected to laboratory testing. The

laboratory samples of lesioned reactor animals usually consist of at least one lesion, whereas for non-lesioned reactor animals the samples are usually the retro-pharyngeal, bronchial and mediastinal lymph nodes which are bulked together by herd. When lesions are negative on histological examination or when no lesions are found, bacteriological culture is performed.

2.3. Study design

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

The net bovine rise was calculated as the increase (in millimetres) at the bovine tuberculin injection site greater than any increase at the avian tuberculin injection site when measured after 72 hours (as per Council Directive 64/432/EEC, Annex B). The risk status of the disclosure test was divided into 3 types: routine (in situations where no risk of bTB infection is suspected to be in the herd), at risk (in situations where the herd is at increased risk of having bTB infection) and restricted (in situation where reactors or animals with lesions at routine slaughter have been found or the herd is at high risk of

having bTB infection). The bTB history in the previous herd was defined as bTB being recorded in at least one herd through which the reactor animal moved prior to being in the disclosure herd and which occurred whilst the reactor animal resided in the herd. Herd size was the average number of cattle tested at herd level tests in the three years prior to the disclosure test. The previous testing history of the reactor animal was taking into account in the analyses by evaluating whether an animal had had an inconclusive (IC) reaction to the SICCT before. An inconclusive reaction to the SICCT can be defined as an increase in skin fold thickness of more than 2 mm and less than 4mm under standard interpretation of the test without diffuse oedema, exudation, necrosis, pain or inflammation of the lymphatic ducts in that region or of the lymph nodes (as per Council Directive 64/432/EEC, Annex B). The previous bTB history of neighbouring herds was taken into account by evaluating whether the animal had a history of being subjected to a Lateral Check Test (LCT) in the previous year. LCTs are carried out on herds that graze land adjacent to that of a herd with a confirmed bTB breakdown. The number of purchased animals was represented by the number of animals entering the herd in the three years prior to the disclosure herd test.

2.4. Data analysis

Microsoft Access 2007 was used for data manipulation while the descriptive analyses were undertaken in 'R' version 2.15.2. (The R Foundation for Statistical Computing). Statistical significance was defined as $p<0.05$. The variables were tabulated and analysis was carried out for each of the variables included in the study. Odds ratios with 95% confidence intervals (95% CI) were calculated to examine the association between the

risk factors and the VL status and bTB status of the reactor animals. The statistical analyses were conducted in GenStat (14.1.0.5943) using generalized linear mixed modeling with the variables herd identifier, local veterinary office (DVO) and abattoir being used as random effects within all the models generated at univariable and multivariable level. The other variables within the dataset were used as fixed effects. Possible first order interactions between the independent variables were explored. The models developed for both VL status and bTB status as being the outcome were firstly checked for significance at univariable level. A *p*-value <0.15 was considered significant. Thereafter a multivariable model of the fixed effects was generated by sequentially adding all the significant variables. This full model was then refined by dropping the non-significant terms with p>0.05 for the final multivariable model.

3. Results

3.1. Missing data

A total of 31,883 reactor animals were present in the database. Complete data on all variables were available for 24,923 reactors (78.2%). Most of the missing data were based on incomplete details on the abattoir the reactor animal was slaughtered in (n=4,895; all in 1998) and missing VL status and/or bTB status (n=1,632) of which the majority (n=1,089; 66.7%) were based on reactors in 1998. Univariable and multivariable analyses were conducted on the reactors (n=24,923) for which all the data were available to be able to take account of abattoir as a random effect. Analyses were repeated on 29,818 reactors for which all data were available except the details of the abattoirs that

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

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

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

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

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

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

the reactor animal was slaughtered in. This analysis was conducted in the same manner as the analysis described previously except that only herd identifier and local veterinary office (DVO) were added as a random variable. This analysis gave similar outcomes to the results described in the current article.

3.2. Dependent variables

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

3.3. Descriptive and univariable analysis

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

3.3.1 Risk factors at host level

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

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

Variable	Reactors %	Visible Lesion Status		bTB status	
	n=24,923	Odds ratio (95%CI)	P value	Odds ratio (95%CI)	P value
Risk factors at host level					
Age at death					
Per10 days increase		0.95 (0.94-0.96)	<0.001	0.95 (0.94-0.96)	<0.001
Breed					
Dairy	45.1%	Baseline	<0.001	Baseline	<0.001
Non-dairy	54.9%	2.29 (2.10-2.49)		2.25 (2.07-2.45)	
Sex					
Bull	3.3%	Baseline	<0.001	Baseline	<0.001
Female	82.1%	1.13 (0.95-1.35)		1.16 (0.97-1.37)	
Bullock	14.6%	1.48 (1.23-1.79)		1.48 (1.23-1.78)	
Test year					
1998	4.5%	Baseline	<0.001	Baseline	<0.001
2002	58.7%	0.53 (0.44-0.64)		0.44 (0.36-0.54)	
2006	36.8%	0.28 (0.23-0.34)		0.25 (0.21-0.30)	
Net Bovine Rise*					
Per 1 mm increase		1.15 (1.14-1.15)	<0.001	1.15 (1.14-1.15)	<0.001
Number of lifetime moves					
0	61.8%	Baseline	0.467	Baseline	0.803
1	21.4%	1.02 (0.97-1.10)		1.02 (0.98-1.10)	
>1	16.7%	0.96 (0.88-1.05)		0.98 (0.90-1.07)	
bTB history in previous herd					
No bTB	18.2%	Baseline	0.085	Baseline	0.200
bTB	20.0%	0.90 (0.81-0.99)		0.92 (0.83-1.01)	
No movement ^	61.8%	N/A^		N/A^	

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

^ 61.9% (n=18,481) of reactor animals remained in their herd of birth until slaughtered and were thus excluded in this part of the analysis.

(Continued on next page)

Variable	Reactors % n=29,846	Visible Lesion Status		bTB status	
		Odds ratio (95%CI)	P value	Odds ratio (95%CI)	P value
Risk factors at host level					
Previous IC [~]					
Not previous IC	96.1%	Baseline	0.130	Baseline	0.177
Previous IC	3.9%	1.13 (0.97-1.31)		1.11 (0.95-1.29)	
LCT [#] previous year					
Not LCT	45.2%	Baseline	0.061	Baseline	0.080
LCT	54.8%	0.93 (0.87-1.00)		0.94 (0.87-1.01)	
Risk factors at test level					
Risk status disclosure test					
Routine	14.7%	Baseline	<0.001	Baseline	<0.001
At risk	29.4%	0.85 (0.76-0.96)		0.87 (0.77-0.97)	
Restricted	55.9%	0.52 (0.59-0.65)		0.52 (0.47-0.58)	
Total nr of reactors					
Per 5 reactors increase		1.04 (1.02-1.06)	<0.001	1.02 (1.00-1.03)	0.059
Risk factors at herd level					
Mean herd size					
Per 10 animals increase		0.98 (0.98-0.99)	<0.001	0.98 (0.98-0.99)	<0.001
Nr purchased in last 3 years					
Per 10 animals increase		1.00 (0.99-1.00)	0.388	1.00 (0.99-1.00)	0.183

[~] Inconclusive

[#] Lateral Check Test

likely to have VL or confirmed bTB than in 1998 (OR for VL=0.53 and 0.28 respectively; OR for confirmed bTB=0.44 and 0.25 respectively).

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

3.3.2. Risk factors at test level

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

3.3.3 Risk factors at herd level

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

3.4. Multivariable analysis

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

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

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

~ Inconclusive

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

4. Discussion

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

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

Several studies (Frankena *et al.,* 2007; Olea-Popelka *et al.,* 2012; Shittu *et al.,* 2013; Wright *et al.,* 2013) described substantial variation in the effectiveness of lesion disclosure and subsequent confirmation of bTB among different abattoirs in the Republic

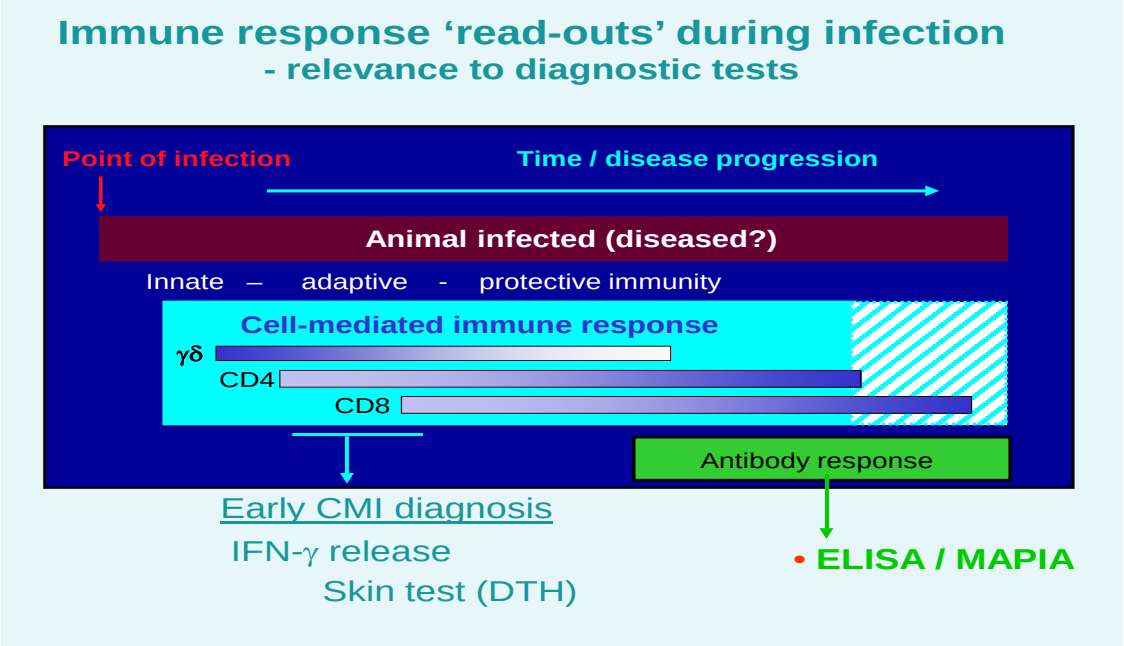


Figure 2: Immune responses in cattle following *Mycobacterium bovis* infection by time/disease progression (McNair *et al.*, 2006)

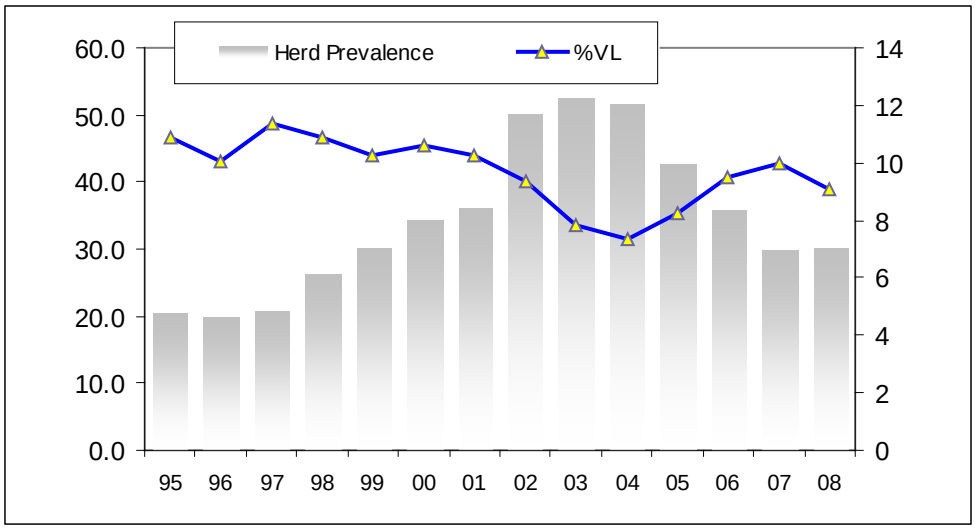
of Ireland, Great Britain and Northern Ireland. This variability was accounted for in the analyses by adding the abattoir where the reactor animals were slaughtered as a random effect at both univariable and multivariable level. In this context it has to be noted that the SICCT is designed to detect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

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

Older reactors were less likely to have visible lesions at post-mortem and confirmed bTB than younger animals. Older animals have had a longer time of possible exposure to *M. bovis* and are therefore more likely to be infected (Griffin *et al*., 1996; DEFRA, 2009), however they might also have a longer time to develop lesion after infection, which would contradict this finding. The difference in visible lesion rate would suggest that there is possibly a difference in the immunological status of younger and older cattle. The immune response of cattle following infection with *M. bovis* is displayed in Figure 2. Early in the bovine life there is an immune responsiveness that is developing and

changing and has had less exposure to infection in general compared to older animals. For example, there are significantly higher numbers of $CD4^+$ T cells present in adult cattle compared to calves (Wilson *et al.*, 1996; Tanaka *et al*., 2006). Furthermore, although bovine neonates cannot produce B cells in sufficient numbers and at birth numbers are very low, by 6 months of age generation of B cells has developed to the adult level (Tanaka *et al*., 2008). Significantly, the bovine immune system, up to 6 months of age, is influenced by the passive uptake of immune regulators acquired through colostrum (Aldridge *et al*., 1998; Barrington and Parish, 2001). These changes in the immune system could create circumstances that may not permit resolution of infection with *M. bovis* in favour of the host.

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



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

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

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

In 2002 and 2006, reactor animals were less likely to have visible lesions or confirmed bTB than in 1998. In 1998, the bTB incidence was rising in Northern Ireland, with a peak incidence in 2002 and decreasing incidence in 2006. In a previous study covering the same time period the visible lesion rate was shown to be converse to the bTB herd prevalence (Figure 3; D.A. Abernethy, pers. comm.). Possibly increased short interval testing due to the increase of bTB breakdowns after 1998 and an increased proportion of animals being removed on severe interpretation has lead to animals having less time to develop visible lesions before being disclosed as reactors.

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

There was no significant association between the number of lifetime moves or the number of animals purchased in the last three years and the VL status and bTB status of reactor animals in this study. The importance of movement and purchase of cattle as a risk factor to bTB infection has been supported by several studies (Gilbert *et al*., 2005; Ramirez-Villaescusa *et al.,* 2009; Ramirez-Villaescusa *et al*., 2010). Abernethy *et al.* (2000) found that there was no significant association between movements through livestock markets and risk of bTB. However these studies have looked at the risk of bTB

breakdown in relation to animal movement and not at the risk of subsequently developing visible lesions.

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

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

after severe interpretation. The same effect may be seen in the total number of reactors at the disclosure test. As the test interpretation changes from standard to severe, more animals in the herd will be reactors based on the SICCT test.

In a previous review by Skuce *et al.* (2011) the most commonly identified risk factors for bTB breakdown were cattle movement, occurrence of bTB on contiguous premises and/or in the surrounding areas, herd size, concurrent disease(s), host genetic variation, immune suppression, age and cattle behaviour. Not all of these risk factors have been addressed within the current study in order to compare these with the risk factors identified in the current study for developing visible lesions or bTB confirmation. However of the risk factors examined, risk factors for bTB breakdown seem to be inconsistent with risk factors for developing visible lesions in most cases. For example increasing age is a risk factor for bTB breakdown (Skuce *et al.*, 2011), but similarly it is protective in relation to the development of visible lesions. This would imply that there are different mechanisms underlying to the risk of becoming infected compared to the risk of actually developing visible lesions.

There is strong evidence from experimental disease models to link the severity of lesion development with the infectious dose administered to cattle (Pollock *et al.*, 2006). Infection doses of 10^8 and 10^7 colony forming units (CFU) administered subcutaneously induced generalised, systemic tuberculosis untypical of field cases of bTB (Francis, 1958; Waddington and Ellwood, 1972). In contrast, in-contact transmission between experimentally *M. bovis* infected and naive cattle resulted in lesion development typically seen in field pathology (Costello *et al.*, 1998). In addition, Buddle *et al.* (1994) stated the cattle infected with a high dose of *M. bovis* (5×10^5 CFU) had more extensive and more

wide spread and larger lesions on post-mortem compared to cattle infected with a low dose of *M. bovis* (5×10^2 CFU). Therefore it is very likely that with respect to the present study, disclosure of visible lesions reflect a stronger infection dose, sufficient to initiate granuloma development.

From a practical view point it is of interest which of these risk factors gives scope in relation to the ability of identification of infected animals. Once we are aware which risk factors are protective (such as increased age) for the purpose of abattoir surveillance more vigilance during the post-mortem examination for animals in these categories could be proposed. Further research is needed into lesion development in cattle especially in relation to their immune response to infection in order to be able to understand the findings in the current study more thoroughly. In addition further research is recommended to evaluate the findings of the current study at herd level.

4. Conclusion

The objective of this study was to get a better insight into the risk factors that affect the development of lesions or positive laboratory tests in bovine tuberculosis reactor cattle in Northern Ireland. Results from this study indicated that a relatively small percentage of reactor animals had visible lesions after post-mortem inspection and/or confirmed bTB by mycobacterial culture. The visible lesion status and bTB status of an animal can affect the way in which bTB breakdowns are managed, since failure to detect visible lesions and recovery of *M. bovis* can lead to a less stringent follow-up after other risk factors have been taken into account. Significant risk factors that increased the risk of visible

lesions and/or confirmed bTB in reactor animals were: a diagnosis made in 1998 (rising bTB incidence), large net bovine skin reaction, a history of being inconclusive at a previous test and increasing number of reactors at the disclosure test. Risk factors that decreased the risk of visible lesions and confirmed bTB were increasing age at time of death, dairy breeds, breeding bulls, being disclosed at a restricted test and increasing herd size of the disclosing herd. These risk factors appear to be related to other factors including the susceptibility and immunological status of the animal.

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References

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

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

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

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

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

Anonymous (2011): Northern Ireland Assembly Committee for Agriculture and Rural Development Official Report (Hansard). Policy and Legislation 7 June 2011. On line available at:

http://archive.niassembly.gov.uk/record/committees2011/Agriculture/110607PolicyandL gislation.pdf. Accessed 29 July 2013.

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

Frankena, K., White, P.W., O'Keeffe, J., Costello, E., Martin, S.W., van Grevenhof, I. and More, S.J. (2007): Quantification of the relative efficiency of factory surveillance in

the disclosure of tuberculosis lesions in attested Irish cattle. Veterinary Record 161, 679-684.

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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