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Author(s): L.E. Green, J.J. Carrique-Mas, S.A. Mason, G.F. Medley
Article Title: Patterns of delayed detection and persistence of bovine tuberculosis in confirmed and unconfirmed herd breakdowns in cattle and cattle herds in Great Britain
Year of publication: 2012

Link to published article:
http://link.aps.org/doi/10.1016/j.prevetmed.2012.04.008
Publisher statement: "NOTICE: this is the author's version of a work that was accepted for publication in Preventive Veterinary Medicine. Changes resulting from the publishing process, such as peer review, editing, corrections, structural formatting, and other quality control mechanisms may not be reflected in this document. Changes may have been made to this work since it was submitted for publication. A definitive version was subsequently published in Preventive Veterinary Medicine, 25th May 2012, DOI: 10.1016/j.prevetmed.2012.04.008

Patterns of delayed detection and persistence of bovine tuberculosis in confirmed and unconfirmed herd breakdowns in cattle and cattle herds in Great Britain

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Abstract

Approximately 1500 / 6000 cattle farms that were depopulated during the foot and mouth epidemic in GB in 2001 had been repopulated and subjected to two unrestricted (herd considered free from bovine tuberculosis (bTB)) herd tests. Factors associated with herd breakdown(s) (HBD) and individual cattle reactor status at the second test were investigated. There were 96 HBD in total, with a three-fold increased risk of HBD in herds that had had a HBD at the first test after restocking. Two mixed effect models were used to investigate factors associated with 324/246060 reactor cattle at the second bTB test; 228 reactors were at confirmed HBD and 96 at unconfirmed HBD; 253 (79%) reactors at the second test that were present and test negative at the first test. In confirmed HBD, the odds of cattle reacting were higher if the restocked farm had a history of bTB before 2001 and if the source and restocked farms were high frequency tested (HFT) farms (routine bTB tests at ≥ 1 per two years). Reacting cattle were more likely to have been born on the restocked farm before the first test after FMD and less likely to have been purchased from a low frequency tested (LFT) farm (routine bTB tests at 3 – 4 year intervals) after the first test compared with a baseline of cattle purchased from a LFT farm before the first test. Unconfirmed HBD at the second test was more likely when the first test was a confirmed HBD and when there was a history of bTB in the restocked farm. In contrast to confirmed HBD, cattle purchased from a LFT farm after the first test were at increased risk of reacting at an unconfirmed HBD at the second test.

We conclude that a farm history of bTB suggests persistence of bTB on the farm. Confirmed tests indicate exposure to bTB for some time indicated by the increased risk from HFT source and restocked farms and a farm history of bTB. The risks for reactors are related to the farm, herd and duration of exposure to those risks. Therefore, the spread of bTB to naïve herds would be reduced if farmers only introduced cattle known not to have been in herds and on

farms exposed to bTB. Management of bTB on farms with bTB is complicated because there is undisclosed infection in cattle and environmental contamination.

Key words: Bovine tuberculosis, herd breakdown, persistence, delayed detection

1. Introduction

In the UK the single intradermal comparative cervical test (SICCT) is used to test cattle for exposure to bovine tuberculosis (bTB). Cattle are given two intradermal injections, one with protein from *Mycobacterium bovis* and one with protein from *Mycobacterium avium*. A herd breakdown (HBD) occurs when, under standard interpretation of the SICCT, there is a relatively larger reaction at the *M. bovis* site of ≥ 5mm skin thickness compared with the *M. avium* site in at least one animal in the herd. These cattle are investigated further for gross lesions indicative of bTB or culture of *M. bovis* and if either further test is positive in at least one reactor then the HBD is confirmed. If a HBD is confirmed, then the cattle are retested and the test is changed to severe interpretation in which cattle are classified as reactors if the skin thickness at the *M. bovis* site is ≥ 3mm more than the *M. avium* site or a swelling of ≥ 1mm at the *M. bovis* site with no reaction at the *M. avium* site. The SICCT test is estimated to be 99.2 – 99.99% specific but a range of sensitivities has been reported from 65% to 95% (Adams, 2001; Costello et al., 1997; Monaghan et al., 1994) and most recently 60% (Clegg et al., 2011). Although the effect of different interpretations of the test have not been quantified, it is likely that the sensitivity of the test is higher and the specificity slightly lower when SICCT results are interpreted under severe interpretation. Once there is a HBD the herd remains restricted until it has one / two clear tests at 60 day intervals for unconfirmed / confirmed HBD, respectively. At this point the herd becomes unrestricted again. One concern about this approach is that the sensitivity os the test is lower in animals that are tested at frequent intervals (Radunz and Lepper 1985; Coad et al., 2010) and consequently a herd might become unrestricted when cattle fail to respond to the test rather than when bTB is eliminated.

The 2001 foot-and-mouth disease (FMD) epidemic in Great Britain (GB) resulted in the depopulation of approximately 6000 cattle farms (Davies, 2002). A large number of these farms were subsequently restocked with cattle and these newly formed herds had a compulsory test for bovine tuberculosis (bTB). By August 2004 approximately 3000 herds had had their first test and 6% of herds had a HBD (Carrique-Mas et al., 2008). The main risks associated with HBD at the first test were purchasing cattle from farms that were tested biennially or more frequently for eight years before the FMD epidemic, a history of HBD with bTB on the farm in the five years before destocking and the number of cattle tested (Carrique-Mas et al., 2008).

Restocked farms in the north of England were primarily only at risk from bTB when they were restocked with cattle from high risk areas (Carrique Mas, 2007). Purchase of infected cattle was, therefore, the most likely explanation for a HBD on these newly restocked farms with no history of bTB. These conclusions have been supported by other studies (Gilbert, 2005; Gopal et al., 2006; Ramirez-Villaescusa et al., 2009; Reilly and Courtenay, 2007; Wolfe et al., 2009).

If cattle are the only source of exposure to bTB for other cattle, then destocking and restocking should have 'reset' all herds to time zero, i.e. there should not be a farm (local) effect nor should risks for previous HBD on the depopulated farm carry over to restocked cattle. The identification of a farm risk separate from a herd risk provides evidence for a farm / environment reservoir which is separate from the cattle infection status. The farm-based environmental risk is likely to be the result, at least in part, of badger (*Meles meles*) infection with *M. bovis*, demonstrated by the reduction in risk when badgers are removed (Donnelly et al., 2006; Griffin et al., 2005) and possibly contamination in the environment (Courtenay, 2006) or slurry (Reilly and Courtenay, 2007) or contact with cattle from neighbouring herds (Johnston et al., 2011).

Results from the first bTB test after FMD in these repopulated herds also indicated an exponential decay in the risk of HBD dependent on the time since the last HBD such that the risk was not detectable if the previous HBD occurred >5yrs before the first test after restocking (Carrique-Mas et al., 2008). This decay has two potential explanations. First, that the risk from local / farm reservoirs decays exponentially, i.e. a real time effect. Second, that this decay is an artefact of the testing regime. The testing interval is associated with the historic farm risk so the last test before FMD in annual or biennial testing restocked farms is likely to have occurred more recently than in 3 or 4 year testing restocked farms. If the environmental risk on farms is heterogeneous and relatively constant, then farms that are tested more frequently would have a higher risk of HBD and the interval between HBD would be shorter, leading to a perceived decay in risk. It is important to distinguish between these explanations: if the first is correct, then it is possible that farms can be cleared of *M. bovis* by exclusion of cattle for a period of time.

Consequently, the principal risks of a HBD are derived from the farm environment (measured as recent history of HBD) and from cattle history (measured as the history of HBD in herds in which cattle have been resident). The risk of an individual animal being a reactor is dependent on the history of the herds in which an individual has resided. In the current study we examine the risks for both HBD and individual cattle being reactors in the restocked herds investigated by Carrique-Mas et al. (2008) at their second unrestricted herd test for bTB. We were specifically interested in whether cattle moved from high frequency tested (annual and biennial tested) herds were still at high risk of reacting to the second test, and wished to further investigate the pattern of risk associated with a farm’s previous history of bTB.

2. Materials and methods

2.1 Data

In 2003, movement data from the cattle tracing system (CTS) and bovine tuberculin testing data from VetNet (Mitchell et al., 2005) were used to construct a dataset of the population of 3000 restocked herds that had been tested for bTB after restocking (Carrique-Mas et al., 2008). In August 2004 the second unrestricted herd test results for 1500 of these herds were added to the database. The outcome of interest was the second unrestricted bTB test after FMD. For herds negative at the first test this was a check test, whole herd test or routine herd test; these are all herd tests that are done on unrestricted herds that have not recently broken down (see Green and Cornell 2005 for details). For herds positive at the first test the outcome of the six-month test after the lifting of movement restrictions was used; this test is only done on herds that have broken down.

We used these 1500 unrestricted restocked herds to investigate the risks for a second HBD and risks for individual animal reactors. For the herds, the variables investigated were a confirmed / unconfirmed HBD at the first test after restocking, herd size, annual or biennial testing (farm had had ≥ 4 herd tests in the previous 8 years) i.e. high testing frequency herds (HTFH) versus 3 or 4 year testing interval, i.e. low testing frequency herds (LTFH) in the restocked herd and source herd and a history of bTB on the restocked farm.

For individual cattle present in the herds at the second unrestricted test, the risk of being a reactor was analysed. The variables investigated were the same as those for the herd. In addition, cattle were classified as purchased before the first test, born in the restocked herd before the first test, purchased after the first test, or born in the restocked herd after the first test. The source and restocked herds were classified as either HFTH or LFTH; cattle born into a subject herd were classified on the basis of that herd. Cattle age was calculated as $\log_{10}$ age in months.

2.2 Data analysis

The relationship between the time since the last HBD before FMD and a HBD at the first and second tests was investigated, using t tests and chi squared tests as appropriate, to determine whether there was a decrease in risk with time since last HBD in previously affected herds.

Two multivariable hierarchical binomial logistic regression models with random effects (Goldstein, 2003) were developed: one with reactors that were identified at confirmed HBD (in which reactors at unconfirmed HBD were coded as missing) and one with cattle that were reactors at unconfirmed HBD (in which reactors at confirmed HBD were coded as missing). It was not possible to define individual reactor cattle as confirmed or unconfirmed because not all cattle are investigated for lesions or culture of *M. bovis* at confirmed HBD. The model hierarchy was level 1 (animal) clustered by level 2 (the source herd) and by level 3 (the restocked herd). For cattle born in the restocked herd, the restocked and source herds were coded as the same herd. The variables listed above were tested in these multivariable models. The goodness of fit of the model was assessed using the Hosmer-Lemeshow statistic (Dohoo, 2003).

3. Results

Of the 1500 herds tested twice by August 2004 after restocking, 1321 were negative on both occasions. Out of 63 unconfirmed first tests, 59, 3 and 1 were negative, unconfirmed and confirmed at the second test, respectively. Out of 50 confirmed tests at the first test 37, 7 and 6 were negative, unconfirmed and confirmed at the second test, respectively. Of the 113 herds with HBD at the first test, 17 (15%) also had a HBD at the second test ($\chi2= 19.7$, $p<0.01$); 13 of these 17 were confirmed HBD at the first test.

There were 230163, 9927 and 5970 cattle in herds that were test negative, unconfirmed and confirmed at the second test, respectively, with 96 (0.1%) cattle test positive at unconfirmed HBD and 228 (3.82%) cattle test positive at confirmed HBD (Table 1). Of the 96 reactors from unconfirmed HBD, 70, 4 and 22 were from herds with negative, unconfirmed and confirmed HBD at the first test, respectively. Of the 228 reactors from confirmed HBD, 191, 7 and 30 were from herds with negative, unconfirmed and confirmed HBD at the first test, respectively.

The mean inter-test interval did not differ between herds that did not and did break down at the first test (12.6 months vs. 12.4 months, $Z=-0.45$, $p=0.66$). Herds in high testing frequency areas were tested at a slightly greater interval than herds in low testing frequency areas (13.2 months vs. 12.2 months, $Z=1.679$;$p=0.11$), but this difference was not statistically significant. Because of the similarity of this time interval between all types of herd, this variable was not included in further analyses.

Cattle purchased before the first test and still on the farm and tested at the second test had a median age of 48 months, whereas cattle born after the first test had a median age of 6 months. Cattle born on the farm before the first test had a median age of 15 months and those purchased after the first test had a median age of 19 months. There was no significant difference in the mean age of cattle from high or low frequency testing herds and they had spent a similar amount of time in restocked herds (data not shown).

In the univariable statistics (Table 1) cattle were at increased risk of being classified as a reactor at both confirmed and unconfirmed tests with increasing age. In unconfirmed breakdowns cattle were at reduced risk of being reactors if they were born on the tested farm after the first test and at increased risk if purchased from a HFTH before the first test compared with cattle purchased from LFTH before the first test. In addition, cattle were more likely to be reactors if the restocked herd was HFTH and if the herd had a history of bTB before 2001. In confirmed breakdowns there was an increased risk of cattle being reactors if the source herd (including the restocked herd for cattle born on the farm) was HFTH and cattle purchased or born after the first test were at reduced risk of being reactors compared with cattle purchased or born before the first test.

The odds ratio (OR) is a magnitude of risk in comparison to a baseline e.g. the risk of an animal being a reactor at an unconfirmed positive second test was 9.27 fold (95% confidence intervals 1.74 – 49.44) if the previous herd test had been a confirmed test (Table 2). From Table 1 we can see that 1.58% cattle were reactors at such a test compared with 0.87% cattle that were in the baseline category (reactors at an unconfirmed test when the first test had been negative) before adjusting for other effects. The confidence intervals indicate that it is 95% likely that the true OR lies between 1.74 and 49.44. These wide confidence intervals indicate large uncertainty in the likely true value of the OR. In this dataset the wide confidence intervals are likely to occur because reactors were rare (324 / 246060 cattle were reactors) and clustered by explanatory variable making some explanatory variables less robust in determining the likely true value of the OR.

Once these variables were combined in the multivariable mixed model, the risks for cattle reacting at confirmed tests were increasing age, being born on the farm after the first test, the restocked and source farms being HFTH and that the restocked farm had a history of bTB before 2001 (Table 2). It can be seen from Table 1 that increasing age was an important crude risk for an animal being a reactor, with the risk of reacting increasing dramatically with age from 0.08% with 2 – 8% cattle above two years of age reacting to the test. In the final multivariable model, after adjusting for other variables, the increasing risk with log age equated to a doubling of risk of being a reactor in a HBD for cattle of 5 years of age versus those of 6 months in both confirmed and unconfirmed tests. There was a reduced risk of cattle reacting if they had been sourced from a LFTH after the first test.

The risks for cattle reacting in unconfirmed tests were increasing age, a confirmed HBD at the first test, purchase from a LFTH after the first test compared with before the first test and a history of bTB before 2001 on the restocked farm. The magnitude of the OR for age was five times greater in cattle reacting at unconfirmed HBD than confirmed HBD. This result is because the baseline risk for young cattle in unconfirmed tests was so low and the OR is relative to the change in risk with increasing age.

The probabilities of a HBD at the second test by HBD at the first test stratified by the last year that that herd experienced a HBD before FMD (Table 3, Figure 1) were calculated. There were small numbers in each category, especially for the herds that had a HBD at the first and second tests. However, in contrast with the marked decay in risk with time since previous HBD observed at the first test (Carrique-Mas *et al.*, 2008), the risk of HBD at the second test was independent from herd bTB history prior to FMD (compare dashed lines in Figure 1).

4. Discussion

Destocking and restocking of herds during the 2001 foot and mouth epidemic in the UK provided a natural experiment to study the risks of HBD with bTB. All the results from this study come from a small amount of data, but these are all the data that we have from restocked farms that arose from this rare event of depopulation and repopulation of 1500 herds. Using mixed effect models enables us to adjust for dependency of cattle within herds, however, a limitation of these models, as with all discrete outcome models, is the approximations used. We have estimated the risks for individual cattle being reactors at the second test following restocking differentiating between cattle detected at confirmed and unconfirmed HBD. Without distinguishing between confirmed and unconfirmed HBD the results from the confirmed HBD dominate (results not shown).

Herd size is frequently reported as a significant risk for HBD, so it is interesting that this variable was not significant in the multivariable models in the current study. Most analyses of risk of HBD have concentrated on herds as the unit of study, which reflects the control programme. However, individual cattle move between herds in the UK at a rate that means herds are not self-contained units, and risks are carried between herds and distributed over time. In a recent analysis of repeated HBD, Karolemeas et al. (2011) also did not report an effect of herd size, suggesting that the multivariable models have explained the risks that are correlated to herd size.

The inter-test interval did not vary between herds that had a HBD at the first test and those that did not; this is most likely because the time from a HBD to removal of restrictions plus time to the first 6 month test was approximately a year, and the herds that did not break down had their second test approximately one year after their first test either because they were in a one year testing area or because they had a further check test after purchasing more cattle.

There were few cattle that were reactors compared to the number tested in the current cohort of farms. The risks identified in the current study might be less confounded than those from studies where herds have been in continuous existence for many decades; no herd in this study was older than 3 years, although some cattle were as old as 8 years.

The greatest risk for cattle reacting to the SICCT in the current study, whether at a confirmed or unconfirmed HBD, was increasing age: there was a 9 - 45 fold odds of reacting with each $\log_{10}$ increase in age in months. The high OR do indicate the dramatic increase in risk of reacting with increasing age, with up to a 50 fold crude risk apparent from Table 1. Age is likely to be a proxy measurement of the combined period of exposure to *M. bovis* and period for development of positive skin reaction after exposure, as well as the number of tests experienced. Since we were unable to disentangle these durations and events we retained age in the analysis. Ideally age would be better explained as durations of exposure and latency to the SICTT test.

In an unconfirmed HBD all reacting cattle are unconfirmed, that is they have no visible lesions or cultures of *M. bovis* and so the standard interpretation of the SICCT (a skin reaction of 5mm or more) is used. In the current study an unconfirmed HBD at the second test was more likely to occur in herds that had had a confirmed HBD at the first test than in herds that were negative at the first test. This might suggest that cattle which had been infected for sufficiently long to develop lesions or to have reduced immune response to the skin test (Radunz and Lepper 1985) were removed at the first test and those that tested positive at the second test were more recently exposed. After adjusting for age, result of the first test and herd history of bTB, cattle purchased from a low frequency testing herd were at increased risk of reacting at this second test. There are several possible explanations for this; one is that these cattle were naïve when they arrived on an infected farm and tested negative at the first test but positive at the second test because of exposure that occurred whilst on the farm, either after the first test or at sufficiently low dose that they tested negative at the first test. Another explanation is that whilst low frequency testing herds are considered at lower risk of having cattle exposed to bTB, some herds will be infected but undetected because they have not been tested for some time because of the long intertest interval. However, cattle from these herds pose a risk if moved in this untested interval (Green and Cornell 2005).

In contrast, there was no association between a confirmed second test and a confirmed first test. Given that both confirmed and unconfirmed HBD were more likely in herds with a history of bTB it does raise the possibility that herds cycle between confirmed and unconfirmed HBD: raising the sensitivity by using severe interpretation removes more infected cattle but is not sufficient to remove bTB from the herd and farm and so infection recrudesces over time, initially with an unconfirmed HBD due to more recent exposure. The unconfirmed HBD does not remove some infected cattle and these are then confirmed at a subsequent HBD.

In addition, the risks of a confirmed test were different from the risks for an unconfirmed test. However, we do not know which of the reactors at the second test were confirmed – some cattle would have been unconfirmed and presumably have had risks similar to the reactors in the unconfirmed HBD model. After adjusting for age, reacting cattle from a confirmed HBD were more likely to have been on the farm at the first test, whether born or purchased (Table

2) than cattle born or purchased after the first test. One explanation for their failure to be detected at the first test is that they were exposed after the first test; another is that they were exposed but missed by the test; 20 – 40% truly exposed cattle would be test negative according to the test sensitivity. A third explanation is that these cattle would have been tested at least once before and that they did react to the skin test but not sufficiently to be reactors under less severe interpretation (Radunz and Lepper 1985), but such cattle were classified as reactors because the test interpretation at the second, confirmed, test was severe. All the other variables associated with cattle being reactors at a confirmed HBD were due to likely persistence of bTB from restocked and source farms with a history of bTB and annual or biennial testing. These patterns of risk for HBD were also reported by Ramirez – Villaescusa et al. (2009).

In this discussion we have assumed that the animal test specificity is 100%, i.e. that all test positive cattle were truly positive. Whilst with increasing numbers of cattle tested even a specificity of slightly less than 100% would lead to some false positives this. However, this appears a rare event: discussions on those modelling bTB conclude that if specificity was much lower than 100% then there would be many more HBD (personal communication Karolemeas). If all reactor cattle were truly infected, and if these cattle had been purchased already infected, then 91 cattle from annual/biennial testing source herds that were reactors at the second test should theoretically have been detected and removed at the first test. If the sensitivity is 60-95% then the expected number of truly infected animals undetected at the first test would be between approximately 5 and 36. Assuming the same sensitivity the number of these animals detected at the second test would be between 5 and 28. There are three possible explanations for many more animals (91) being detected at the second test. First, there was an increase in the number of infections between tests; second, test sensitivity changed between the two tests and third, the test has low sensitivity in the field and many infected cattle were not detected at the first test.

These results are consistent with a less than perfect sensitivity of the test, which is a critical limitation for the control of bTB in GB (Green and Medley, 2008) and elsewhere (de la Rua-Domenech et al., 2006). It is inevitable that some cattle purchased at restocking were infected but tested negative at the first test, and it is possible that some of these were infectious and contributed to the persistence of *M. bo*vis in the herd. Another plausible explanation for the failure to detect infection at the initial test after restocking would be a longer latent period than previously estimated. The latent period comprises an ‘unresponsive’ or ‘anergic’ period, followed by a ‘reactor period’; the skin test response only occurs in cattle in the reactor period (Neill et al., 2001). The length of this period is not well established. Although Francis (1947) estimated it at 30-50 days, it has been shown experimentally that the development of skin reactivity depends heavily on the initial dose of *M. bovis* (Neill et al., 1991). In experimental studies in which animals were inoculated with high doses of *M. bovis* (i.e. over 104 cfu) intra-nasally or via the tonsils there was development of skin reactivity within 10 weeks (Costello, 1998; Neill et al., 1988; Palmer et al., 2004). However, when cattle were inoculated with low doses the progression to skin reactivity had not occurred by nine months in some cattle, even though limited shedding and limited serological response was reported (Costello, 1998; Neill et al., 1988). Studies with naïve and infected cattle housed together are more likely to resemble natural infection where low-level exposure occurs over a longer period. In one such study the skin reactivity of two of the four animals that tested positive developed after one year (Costello, 1998). Additionally, the effect of continuous or multiple exposures, and indeed multiple testing of cattle, is unknown. It is therefore highly likely that the time to development of response to the skin test varies and that a proportion of cattle develop a response after a long period of time. If these cattle are infectious they might have a disproportionate effect on dissemination of infection. In Scotland, where pre- and post-purchase tests have been carried out on cattle imported from Ireland, more cattle have reacted at the post-purchase test than at the pre-purchase test (Blissit, 2006). This also supports the hypothesis that reactivity following natural exposure may develop over a long period of time, at least several months.

As in previous studies, there was a strong correlation between the outcome of consecutive bTB tests (Olea-Popelka et al., 2008; White and Benhin, 2004). In all cases a history of bTB was the greatest single predictor of HBD at herd level. This was observed in all farms at both the first and second test (Table 2). The time decay in the risk associated with a HBD before FMD that was observed in restocked herds tested immediately after FMD (the first test) but not by the second test does suggest that infection remains in the farm environment for a limited period of time. The period without cattle allowed the decay in environmental risk to be observed directly, and this has been reset by the change in status due to restocking. Had the risk pattern with past HBD remained at the second test, it would have indicated that the pattern was an artefact of the correlation between risk and testing frequency. Consequently, we can conclude that the removal of cattle from these herds did reduce the local risk of HBD, i.e. removing cattle did reduce the future risk of HBD for the farm. It also suggests that the farm environment remains an infection risk for a period of time greater than the period for which these farms were destocked (3 to 12 months).

In conclusion, it is likely that the SICCT test does not detect and eliminate infection in all bTB positive herds in one HBD and that there is residual infection in the herd. This is dramatically evident in the case of restocked herds after FMD, a large proportion of which were restocked with cattle from unrestricted but previously bTB positive herds. The results from this study and that from the first test after restocking (Carrique Mas et al., 2008) do indicate that bTB is spreading into naïve herds in England as a result of introduction of cattle from herds with a history of bTB. This is externally validated by other authors (Gilbert, 2005; Gopal et al., 2006; Ramirez-Villaescusa et al., 2009; Reilly and Courtenay, 2007; Wolfe et al., 2009). An important consequence of the results of this study and others is that farmers, veterinarians and policy makers must appreciate the risks from purchasing cattle from herds with a history of bTB, even if they have not recently had a HBD, versus known free from bTB. This is a concern even when these cattle have passed a bTB skin test. This is particularly important now that there is pre-movement testing for bTB because some farmers believe that this means that tested cattle are definitely free from bTB (Enticott, 2009). A clear method to prevent introduction of bTB into naïve herds is to prevent movement of cattle previously exposed to bTB (Ramirez-Villaescusa et al., 2009) or with unknown history (current paper). This requires that potential purchasers have reliable information about bTB history of herds and individual cattle over many years so that they can make informed decisions.

Acknowledgements

The authors thank Alan Aldridge at CTS and Andy Mitchell at VLA for supplying cattle movement and bTB testing data respectively. This research was funded by DEFRA Project Number SE3026.

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Table 1. Number and percent of 246060 cattle from 1500 herds that were reactors and non reactors by test type negative, unconfirmed and confirmed at the herd's second bovine tuberculosis test in England, 2002-2004 (Test B) by explanatory variables

Explanatory variables	Cattle at Test B							
	Negative bTB tests	Unconfirmed bTB test			Confirmed bTB test			Total no. cattle
	Total number	No. reactors	Total number	% reactors	No. reactors	Total number	% reactors	
First test after restocking (Test A)								
negative	210184	70	8013	0.87	191	4767	4.01	222964
unconfirmed	12351	4	524	0.76	7	381	1.84	13256
confirmed	7628	22	1390	1.58	30	822	3.65	9840
Origin of cattle								
born after test A	61660	2	2284	0.09	10	1711	0.58	65655
born before test A	17467	1	587	0.17	37	352	10.51	18406
purchased LFTH after test A	37522	26	2009	1.29	13	790	1.65	40321
purchased LFTH before test A	80012	31	3117	0.99	63	1093	5.76	84222
purchased HFTH after test A	10311	6	540	1.11	14	597	2.35	11448

purchased HFTH before test A	23191	30	1390	2.16	91	1427	6.38	26008
History of bTB before FMD								
unknown	8229	2	418	0.48	22	196	11.22	8843
no	196308	64	8159	0.78	118	4035	2.92	208502
yes	25626	30	1350	2.22	88	1739	5.06	28715
HFT restocked herd								
no	189347	65	7800	0.83	146	3036	4.81	200183
yes	40816	31	2127	1.46	82	2934	2.79	45877
HFT source herd								
unknown	3138	2	103	0.19	20	101	19.80	3342
no	179227	56	7319	0.08	87	2852	3.05	189398
yes	47798	38	2505	0.15	121	3017	4.01	53320
Age in years (20548 unknown)								
>1	72631	2	2507	0.08	10	1852	0.54	76990
2	47747	7	2317	0.30	29	1277	2.27	51341
3	22009	10	1211	0.83	34	519	6.55	23739
4	24132	15	1156	1.30	38	688	5.52	25976
5	21456	17	838	2.03	21	578	3.63	22872
6	12860	12	590	2.03	21	259	8.11	13709

7+	8082	17	464	3.66	13	323	4.02	8869
Total	230163	96	9927		228	5970	3.82	246060

No. = number, % = percent, Test A = first test after restocking, Test B = second herd test after restocking, bTB = bovine tuberculosis, LFTH = low frequency tested herd, HFTH = high frequency tested herd, FMD = destocked because of the 2001 epidemic of foot and mouth disease

Table 2. Univariable and multivariable odds ratios and 95% confidence intervals for risks for bovine tuberculosis at the second herd test for 228 confirmed reactors and 96 unconfirmed reactors out of 246060 cattle from 1500 herds in England, 2002-2004

Exposure	univariable						multivariable					
	confirmed			unconfirmed			confirmed			unconfirmed		
	OR	95% CI		OR	95% CI		OR	95% CI		OR	95% CI	
Log age in months	14.91	8.92	24.92	45.74	17.61	118.81	9.00	4.14	19.55	48.81	14.54	163.90
bTB at Test A - no	1.00	1.00	1.00	1.00	1.00	1.00	-	-	-	1.00	1.00	1.00
Unconfirmed	0.77	0.05	11.26	2.10	0.39	11.40	-	-	-	1.65	0.28	9.82
Confirmed	3.36	0.20	57.18	9.27	1.74	49.44	-	-	-	9.11	1.57	52.73
History of cattle												
Purchased from LFTH herd before Test A	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Born on farm after test A	0.05	0.02	0.10	0.10	0.03	0.37	0.45	0.19	1.10	1.60	0.37	6.97
Born on farm before test A	1.29	0.81	2.06	0.20	0.03	1.16	2.81	1.60	4.92	1.01	0.20	5.10
Purchased from LFTH herd after Test A	0.17	0.10	0.31	1.40	0.72	2.70	0.42	0.23	0.76	2.60	1.35	4.99

Purchased from HFTH herd after Test A	0.23	0.09	0.59	0.99	1.18	0.84	1.83	0.84	3.97	0.91	0.31	2.64
Purchased from HFTH before Test A	1.03	0.64	1.68	4.13	2.04	8.37	0.89	0.57	1.38	1.75	0.86	3.59
Restocked farm is in HFTH - no	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	-	-	-
Restocked farm is in HFTH –yes	4.01	0.93	17.32	2.78	1.07	7.27	5.79	1.49	22.47	-	-	-
History bTB in restocked herd – no	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
History bTB in restocked herd –yes	1.91	0.97	3.78	2.83	1.30	6.20	1.57	1.04	2.37	3.45	1.81	6.59
Source farm is in HFTH –no	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	-	-	-
Source farm is in HFTH –yes	1.83	1.03	3.26	1.96	0.97	3.98	5.73	1.53	21.41	-	-	-

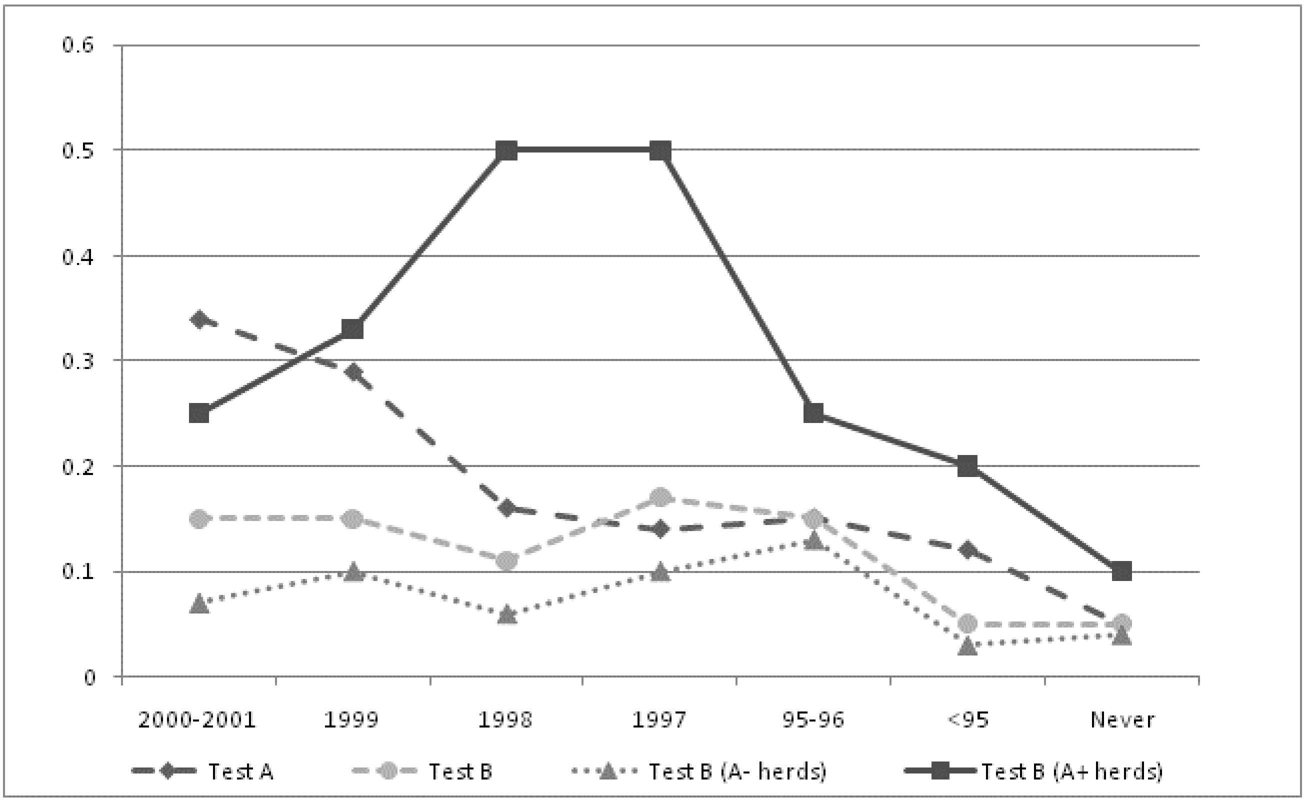
bTB = bovine tuberculosis, Test A = first herd test after restocking, Test B = second herd test after restocking, LFTH = low frequency tested herd, HFTH = high frequency tested herd, - = not significant in multivariable model, 1.00 = baseline risk

Table 3. Risk of herd breakdown (HBD) with bovine tuberculosis at first (Test A) and second (Test B) herd tests after restocking by time since last HBD before destocking because of foot and mouth disease in 2001 in 1500 herds in England, 2002-2004

Year of Last HBD	First test, Test A			Second test, Test B, in herds that did not breakdown at first test (A)			Second test, Test B, in herds that did breakdown at first test A		
	No. Pos.	No. Tested	Risk HBD	No. Pos.	No. Tested	Risk HBD	No. Pos.	No. Tested	Risk HBD
2000-2001	20	59	0.34	1	15	0.07	3	12	0.25
1999	12	42	0.29	2	20	0.10	2	6	0.33
1998	6	37	0.16	1	17	0.06	1	2	0.5
1997	3	22	0.14	1	10	0.10	1	2	0.5
1995-1996	5	34	0.15	2	16	0.13	1	4	0.25
Before ’95	6	52	0.12	1	32	0.03	1	5	0.20
Never	125	2695	0.05	57	1275	0.04	8	82	0.10
Total	177	2941	0.06	65	1385	0.05	17	113	0.15

HBD = herd breakdown, No. = number of herds, Pos.= Positive

Figure 1. Risk of herd breakdown with bovine tuberculosis since last herd breakdown by first and second bTB tests after restocking in 1500 herds England, 2002-2004



Test A = first test after restocking, Test B = second test after restocking, Test B (A- herds) = Test B result for herds that did not breakdown and Test A, Test B (A+ herds) = herds that were tested at B that had had a herd breakdown at Test A.