Title: Factors associated with the risk of, and animal-level response to, M. bovis in Irish cattle, 1993-98

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**Introduction**

In Ireland, the detection of tuberculosis in cattle is heavily dependent on testing individual cattle with the single intradermal comparative tuberculin test (SICTT). Although the control of tuberculosis is focused more at the herd level, we believe it is important to identify and summarise some of the risk factors for bovine tuberculosis at the animal level. The major outcomes in our analyses were the risk of becoming a standard reactor, the skin change (response) to the SICTT, the percentage of SICTT standard reactors among all reactors, and the tubercular lesion risk at slaughter. The risk factors considered included: year, month, county or area (represented by the District Veterinary Office (DVO)), class of animal (cow, heifer, steer, bull), breed type (Friesian versus other), factory (slaughterhouse), and reason for the test (test type). Data on DVO and factory are not presented in this paper, but their effects were controlled when necessary in our analyses.

Although the sensitivity and the specificity of the SICTT are imperfect (neither are 100%; Costello *et al.*, 1997), currently there is no single test that will outperform the SICTT in the total control programme. While acknowledging that not all cattle infected with *M. bovis* have a skin response that would meet the criterion of being a reactor, and that some cattle with a large skin response are not infected with *M. bovis* (Costello *et al.*, 1998), the single most important cause of the skin change in cattle is exposure to *M. bovis*. Unfortunately the sequence of events that takes place subsequent to exposure to this agent varies widely, both in the time taken to become ‘sensitised’ and produce a meaningful skin change, and in the development of readily detectable lesions and shedding of the organism.

In this study, since the timing and extent of exposure to *M. bovis* were not directly measurable, we attempted to identify those factors that were associated with the risk of being a standard reactor, with the skin change, with the proportion of standard reactors among all reactors, and with the detection of lesions at slaughter. We believe this knowledge should be useful in improving our understanding of the current testing programme in Ireland. Our major refinements over routinely collected statistics included using formal measures for risk of a standard reactor (as opposed to apparent prevalence per thousand animal tests (APT)), and the use of multivariable analyses to remove confounding of the outcomes mentioned above.

**Materials and Methods**

The data base containing the results of herd and animal tests was constructed by pooling the data from all 28 DVOs into one central database using Microsoft ACCESS®. Cattle with a skin change where the bovine response is >4 mm more than the avian response are denoted as standard reactors. Other cattle also may be designated as reactors by a veterinary officer with a lesser skin response; usually these are cattle, in the same herd as standard reactors which are considered likely to have been exposed to *M. bovis*. All reactor cattle are identified and slaughtered under permit, and all carcasses, including

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reactor and clear cattle, are examined post-mortem for evidence of gross lesions consistent with tuberculosis at the slaughterhouse.

The reason for testing the animal or herd has a major impact on the results of testing. Briefly, all herds of cattle that are not subject to being tested because of a suspicion of tuberculosis being present are tested once annually on a “round” test; this represents the great majority (~95%) of herds in Ireland in a given year. All cattle in other herds may be tested, one or more times per year, because of:
- tuberculosis in a neighbouring herd (contiguous test),
- a lesion found in a clear animal at routine slaughter (factory lesion test),
- efforts to clear an infected herd (reactor retest)
- efforts to ensure a previously infected herd is clear of infection (six month test)
- as part of the investigation of suspect herds (special check test).

Partial herd tests included the private or pre-movement test and the inconclusive animal retest (cattle that had an inconclusive reaction to the SICCT in an otherwise clear herd are re-tested in 60 days).

**Definition of Outcomes**

The risk of being a standard reactor (STDRCT) was the total number of reactors, with a skin change greater than 4 mm, on that test type divided by the number of animals tested. For risk of being a standard reactor, (i.e., the probability of being a standard reactor) the number of animals tested was the denominator. For the round test, one test per calendar year was assumed and the number tested was obtained directly from the herd-test file. For all other full herd tests the number of cattle tested was the maximum number tested in a single test in that herd under that test type during that year. For private and inconclusive tests the number tested was the sum of all animals tested under that test type in that herd during that year. These files were created in SAS® (SAS/STAT Users Guide, Version 6, Fourth Edition, Cary NC; SAS Institute Inc, 1989).

The SICCT skin change is the difference in skin response to the bovine and avian tuberculin antigens, having adjusted for the initial skin thickness (i.e. \{bovine 72 h response mm – bovine 0 h response mm\} minus \{avian 72 h response mm – avian 0 h response mm\}).

The percentage, of all reactor cattle that were standard reactors was the total number of reactors, with a skin change greater than 4 mm, per 100 reactor cattle. The lesion risk was the total number of cattle with a tuberculous lesion per 100 reactor cattle. The incidence/prevalence risk of a standard reactor was expressed per 1000 cattle tested.

**Statistical Modelling**

Data from the databases were transferred to SAS files (SAS/STAT Users Guide, Version 6, Fourth Edition, Cary NC; SAS Institute Inc, 1989) and analyzed using a variety of procedures but mainly multiple linear regression and multiple logistic regression (using Procedures REG, LOGISTIC and GENMOD). In this database, because of its size, all concerns about ‘power’ of the models are trivial, as almost all factors were ‘statistically associated’ (p<0.05) with the outcomes. Thus the more important question related to the unbiased magnitude of the effects in the multivariable models.

**Results**

The database had data on the response of 197,079 reactor cattle in the years 1993-98. The number of reactors was greatest in 1998 across all classes of cattle. Cows were the predominate class of reactor cattle. The percentage of all reactors that were standard reactors varied between 67% and 70%. The percentage of all reactors with tuberculous lesions ranged from 27 to 37%; it was lowest in 1998 having decreased dramatically in 1996, and again in 1998. The percentage of standard reactors with...
tuberculous lesions ranged from 34 to 44% with a similar annual pattern to “all” reactors.

Numerically, the greatest number of reactors were found on the reactor retest, than the round test, followed by the contiguous herd test. Private tests did not detect more than a small percentage of the total reactors.

Risk of a standard reactor
Over $21 \times 10^6$ cattle-years of testing were conducted on the annual round test and over $23 \times 10^6$ cattle-years of testing were conducted on all other test types combined over the six year period. As the number tested on the round test increased, the number tested on all other test types decreased ($r = -0.95$). Using our approach to estimating denominators, about 7.5 million cattle were tested per year.

The risk of being a standard reactor varied by class with cows having the highest risk followed by steers, then heifers and bulls with the lowest risk. The risk per 1000 animals tested was lowest on the private animal sale test ($0.83/10^3$), for full herd tests it was lowest on the round test ($1.5/10^3$). The risk varied greatly across the other test types; from lowest to highest risk these are, special check test ($1.8/10^3$), six month test ($2.5/10^3$), contiguous test ($2.9/10^3$), reactor retest ($6.5/10^3$), factory lesion test ($7.1/10^3$), and inconclusive retest ($91.9/10^3$).

The annual, monthly, and class risks, on the round test, changed little (<10%) between the crude and adjusted levels—the latter accounting for year, month, class and DVO. Bulls and steers had about the same risk (odds ratio =1), heifers about 15% lower and cows had a significantly (38% higher) greater risk than steers. Seasonally, the risk was lowest in the first 5 months of the year with a nadir in March and a peak in the July through September period, at about 26-30% higher than in the referent month, December.

As shown in Figure 1, the long term trend in risk, on the round test, appears to be towards a slightly increased risk. The clear seasonal pattern with the highest risks in the last half of most calendar years is evident. This pattern was similar over the four classes of cattle. Because of repeated testing within the year, seasonal patterns for the other (non-round) test types were not investigated. However, as one comparison for seasonal results, we computed the risk of being a standard reactor on the contiguous test. The results were very similar with a peak period of risk for the months of July through October with odds ratios of 1.21, 1.3, 1.38 and 1.2 respectively, relative to December.

**Figure 1.** Crude risk of standard reactor, on the Round Test, in Ireland, 1993 – 98.
Skin change

The distribution of SICTT skin change was highly skewed, with a mean of 10.9 mm and a median of 7.5 mm on the round test; 1.2% of the responses were negative, meaning that the response to the avian tuberculin was greater than that to the bovine tuberculin, while 22% of responses were greater than 22 mm. The modal response on the round test was 5 mm in cows and 6 mm in other classes; responses above 4 mm qualify the animal as a standard reactor. The overall median response in cows was 3 mm and 4.5 mm in other classes (Figure 2). Note that cows have a narrower band (variance) and a lower mean than other classes.

Figure 2. Skin changes in Irish Cattle 1993 – 98.

There was considerable variation in skin response across test types. On average, relative to the round test results, the results of inconclusive re-tests were 4.5 mm smaller, reactor retests were 2.3 mm smaller, six-month tests were 1.3 mm smaller, special check tests were 0.8 mm smaller, contiguous and factory lesion tests produced similar responses, whereas private test results were 1.1 mm higher. Over the period 1993-98, the crude skin change was moderately stable. The response of Friesians was more variable seasonally, than that of non-Friesian cattle.

There was little variation in skin response across months; most differences were less than 1.5 mm. The adjustment of the seasonal pattern for the presence of lesions greatly reduced the skin response in March, but increased the skin response in the July-October period.

Percent Standard Reactors

Approximately 70% of all reactors, on the round test, were deemed to be standard reactors; this was moderately constant in the period 1993–98. There was little change in the percent standard reactors on the non-round test data in the years 1994 to 1998. The monthly crude proportions of standard reactors were higher in the first 6 months of the calendar year; this was also true when the adjustment for class, Friesian and lesion were made. As per the skin change, cows had the lowest proportion of standard reactors, maiden heifers the highest. Friesians had a lower crude proportion of standard reactors; however, the crude proportion was highly confounded largely by class of animals, as per the skin change.

Risk of Lesion

The risk of disclosure of a lesion of tuberculosis at slaughter varied from 33.6% to 43.6% in standard reactors and 26.6% to 36.6% in all reactors. The risk also varied with the slaughterhouse (factory) and hence adjustments for this are necessary in order to make valid comparisons. Part of this effect reflects
the type of cattle they receive; however, the majority of the between factory variance in lesion risk appears to relate to the factory itself.

Based on the data shown in Figure 3, the annual lesion risk was highest in the 1993-1995 period and dropped markedly in 1998 for cattle tested on the round test as well as all non-round tests. The crude annual risks were moderately confounded, with the adjusted risks being much more variable than the crude risks.

Seasonally, the risk of a lesion was much higher in the winter and early spring period, an exception being cattle slaughtered in February. Reactors slaughtered in July through September had the lowest risk of a lesion. The crude risks were not confounded.

**Figure 3.** Crude Lesion Risks in Standard Reactor Cattle (1993 – 98).

Cows had the lowest crude risk of a lesion followed by steers and bulls; maiden heifers had an elevated crude risk. After control of potential confounder variables, cows and bulls had a similar risk followed by steers. With skin change controlled, bulls had the lowest risk while cows and steers had similar risks.

The adjusted lesion risks for standard reactors detected on the round test, after adjustment for class, DVO and factory, are shown in Figure 4. Note the regularity of the seasonal effect on lesion risk. The decreasing lesion risk in standard reactors during the 6 year period is also apparent.
Since skin change is a major predictor of finding a tuberculous lesion we adjusted the lesion risk for skin change in addition to the other factors. When this was done there was virtually no change in the seasonal pattern of risk; similarly, the adjusted annual risks changed very little. Surprisingly, the difference in lesion risk by class persists even after adjusting for skin change. At any given skin change, cows have a 30-36% lower risk of disclosing a lesion than a heifer and 19% lower than a steer.

Discussion
The data used in this study reflect the events that occurred over a 6-year period in the bovine tuberculosis eradication programme in Ireland. Understanding the events or attributes related to the risk of tuberculosis, including its detection, at the animal level is an important step before proceeding to examine patterns at the herd level.

Based on transmission studies using reactor and susceptible cattle housed together, the general rule is that transmission, as determined solely by reactivity to the tuberculin test, occurs only at low levels over a protracted period. A second general rule is that the temporal and biological response is proportional to the dose. Large doses lead to more extensive lesions earlier post-exposure. Recent studies with low exposure doses in calves indicate that the calves respond over an 18-month time frame. Experimentally challenged calves show a positive gamma-interferon response within 4 weeks of exposure (Buddle et al., 1995).

The underlying risk of becoming a standard reactor is a parameter of great importance but it is not frequently determined because of the need to record the denominator data and integrate these with the numerator (reactor) data. Seasonally, the greatest risk was experienced in the summer and early fall. The seasonal results on the contiguous test were very similar to those on the round test, and this would tend to rule out a seasonal bias towards testing clear herds in order of estimated risk and support a seasonal exposure to M. bovis. The classes of cattle differed in their risks, which we infer represents either a different level of exposure by class, or a different ability to respond to exposure. Cows had the highest risk of becoming a standard reactor despite their tendency to have smaller skin changes than other classes. In contrast, heifers had the greatest skin changes but the lowest risks.
Since the skin change to the SICCT is the major bovine tuberculosis detection method used by veterinarians, this phenomenon is a logical place to start to understand the factors influencing the detection of tuberculosis in Irish cattle by means of the tuberculin test. The skin response, in reactor cattle, is very skewed but the vast majority of reactor cattle have responses in the 2 to 14 mm range; >4 mm being the skin change cut-point to define a standard reactor. Skin responses in “clear” cattle were not studied but in any case they would be less than 3 mm.

Overall there is little evidence of a major long term trend in skin change; nor is there evidence of marked short term changes. Since cattle with lesions tend to have larger skin changes, adjusting skin change for the presence of a lesion tends to reduce the effect of year for those years with relatively more lesions.

Interestingly, the classes of animals have a different average skin change, and we infer that this may be a function of age since cows have the smallest skin change, followed by bulls, while the younger classes (heifers and steers) have a larger skin response. Since an animal’s response is proportional to the exposure, this increased response in younger classes of cattle could be due to an extremely high level of exposure or due to a stronger immune response to the same level of exposure.

There is a large difference in skin change across the various reasons for testing the cattle and hence most comparisons need to control for this, or just focus on one test type. We have tended to use the round test results as our basis since over 90% of the animals tested in a given year are tested for this reason. Virtually all “other test types” had smaller average skin changes than cattle on the round test; however, it is difficult to make other causal inferences based on the differences by test type. The percentage of standard reactors had a much more pronounced seasonal pattern than skin change; the greatest levels being obtained in the months February to June. This seasonality could reflect a seasonal exposure or a seasonal immune response. Anecdotal evidence suggested that cows would have a reduced skin change in the early spring months because of the stress of calving. However, there is no support for this in the data used here. We infer that it more likely reflects an increased level of exposure in the preceding months.

Lastly, the risk of a lesion also differed according to a number of factors. Unlike the relatively stable skin change over the years, and the high risk of being a standard reactor in 1998, the risk of a lesion was lowest in 1998. Thus, we accept that either a larger proportion of the reactor cattle are not really infected with *M. bovis*, in the later study years, or if they are infected they are early post-infection and therefore were less likely to display lesions at slaughter.

Seasonally, the risk of a lesion increased from its nadir in the July-September period to a maximum in March before starting to decline again. While this coincides with the cattle housing period in most of Ireland, the increases in lesions in October and November occur very shortly after the housing period would normally begin. Hence a cause and effect relationship may not be present. Whatever the cause however, the lesion risk is very consistently seasonal. Cows had the lowest risk, followed by bulls, with the younger classes of cattle having a higher risk of a lesion. This ordering persisted even when the skin change was controlled. This may indicate that young animals have an exaggerated response relative to cows, and despite this larger skin response they appear to be less able to control the original infection and prevent detectable lesions from developing.
Thus it would appear that cows are more likely exposed to *M. bovis* than other classes of cattle, but when exposed they have a lesser skin response and a smaller chance of having a lesion at slaughter. On the other hand, heifers (and steers) have a lower risk of exposure but when exposed they have a more marked skin response and a higher risk of having a lesion than cows. These class differences need to be born in mind when interpreting data; most experimental work is done using calves, a class that may be relatively “hypersensitive” in the timing of, and extent of, response to challenge.

Temporal changes can often provide clues as to causal factors. In this regard the annual variations in risk are very marked. Some of these undoubtedly relate to explicit programme changes, but explanations for other annual variations are not as readily available. The decline in lesion risk in the last 5 years of the study period also is not explained by either changes in risk or in physiologic response. On the seasonal side, the skin change (reflecting physiologic response to exposure) changed very little was stable over the year, but with a tendency towards a greater response in the months of February through June. However, the risk of being a standard reactor was greatest in the second half of the year. If developing a lesion has a lag period of at least 2-3 months after field exposure, then this might explain the seasonal aspect as the risk of exposure being detected by means of the tuberculin test began to peak in July and persisted until December or January. The time lag between detection and actual exposure is unknown but is likely to be a matter of a few months, thus we posit that the actual exposure would begin to increase early in the spring about the time cattle are let out to grass. In order to explain the class risk differential, one would have to posit that cows are exposed to more “outdoor” activity sooner than other classes of cattle. Note that this scenario does not contradict the long held belief that housing of reactor cattle with susceptible cattle increases the risk of transmission. Rather, it seems on balance that the testing programme removes the highest risk cattle earlier in the year. Animals that were infected earlier develop lesions during the winter feeding period, but as has been shown in many studies, in most instances the apparent rate of spread from these reactors is low.

References

