





Bovine tuberculosis

Tuberculosis in cattle

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Improving surveillance

Field-based surveillance

Using latent class analysis to estimate the test characteristics of the interferon- γ test, the single intradermal comparative tuberculin test and a multiplex immunoassay under Irish conditions

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Veterinary Microbiology 151, 68-76 (2011)

Considerable effort has been devoted to improving the existing diagnostic tests for bovine tuberculosis (single intradermal comparative tuberculin test [SICTT] and γ -interferon assay [γ -IFN]) and to develop new tests. Previously, the diagnostic characteristics (sensitivity, specificity) have been estimated in populations with defined infection status. However, these approaches can be problematic as there may be few herds in Ireland where freedom from infection is guaranteed. We used latent class models to estimate the diagnostic characteristics of existing (SICTT and γ -IFN) and new (multiplex immunoassay [Enferplex-TB]) diagnostic tests under Irish field conditions where true disease status was unknown. The study population consisted of herds recruited in areas with no known TB problems (2,197 animals) and herds experiencing a confirmed TB breakdown (2,740 animals). A Bayesian model was developed, allowing for dependence between SICTT and γ -IFN, while assuming independence from the Enferplex-TB test. Different test interpretations were used for the analysis: SICTT (standard and severe interpretation), γ -IFN (a single interpretation), and a range of interpretations for the Enferplex-TB (level-1 [high sensitivity interpretation] to level-5 [high specificity interpretation]). The sensitivity and specificity (95% posterior credibility intervals; 95% PCI) of SICTT[standard] relative to Enferplex-TB[level-1] and γ -IFN were 52.9-60.8% and 99.2-99.8%, respectively. Equivalent estimates for γ -IFN relative to Enferplex-TB[level-1] and SICTT were 63.1-70.1% and 86.8-89.4%, respectively. Sensitivity of Enferplex-TB[level-1] (95% PCI: 64.8-71.9%) was superior to the SICTT[standard], and specificity of the Enferplex-TB[level-5] was superior to γ -IFN (95% PCI: 99.6-100.0%). These results provide robust measures of sensitivity and specificity under field conditions in Ireland and suggest that the Enferplex-TB test has the potential to improve on current diagnostics for TB infection in cattle. The extent of that potential will be assessed in further studies.

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A follow up study of the γ -interferon test results from the latent class analysis

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The objective of this study is to identify risk factors associated with animals falsely testing positive to the gamma-interferon test. The study will follow animals from a very low bTB prevalence area that were tested using gamma-interferon in 2008. These animals were followed until the end of 2010 to identify any that were 'true' bTB positives in the follow-up period. Animals that were found to be bTB negative will be examined to identify risk factors, such as age or location, that may be associated with testing (falsely) positive to the gamma-interferon test in 2008.

Primary isolation of *Mycobacterium bovis* from bovine tissues: Conditions for maximising the number of positive cultures

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Veterinary Microbiology (in press)

In studies of *Mycobacterium bovis* infection in animals a definitive diagnosis requires the isolation of the organism. However, the optimum conditions for the primary isolation of *M. bovis* have not been determined. The aim of this study was to determine for primary isolation of *M. bovis*, (a) the incubation time required to achieve maximum sensitivity (i.e., the number of positive samples identified), (b) the effect of decontaminants on bacterial growth rates, and (c) the influence of media and the number of slopes of media on the number of positive samples detected. Two agar-based media, modified Middlebrook 7H11 (7H11) and tuberculosis blood agar (B83), and an egg-based medium, Stonebrink's (SB) were compared. Three decontaminants, 2% (w/v) sodium hydroxide (NaOH), 0.75% (w/v) and 0.075% (w/v) cetylpyridinium chloride (CPC, also called hexadecylpyridinium chloride, HPC) and 0.5% (w/v) benzalkonium chloride (BC) were evaluated against treatment with sterile distilled water. The inoculated media slopes were incubated for up to 15 weeks. Colonies first appeared after 2 weeks on all media types and 75% of positive slopes were identified by 8 weeks. An incubation time of 15 weeks was required to identify all positive samples. The slowest growth was associated with inocula that contained the fewest viable bacilli. The time to the appearance of colonies was influenced by medium type: the median time to detection of colonies was 28 days on 7H11 and B83, and 36 days on SB. However, SB returned the greatest number of positive samples. Decontamination procedures increased the minimum incubation time required to detect positive cultures, probably due to the toxic effect of the decontaminants. Increasing the number of inoculated slopes resulted in an increased number of positive samples and a decreased time to the detection of colonies. Overall, the detection of *M. bovis* was significantly influenced by the choice of media, the decontaminant and the duration of incubation of cultures.

Reprinted from Veterinary Microbiology, Corner, L.A.L., Gormley, E., Pfeiffer, D.U., Primary isolation of Mycobacterium bovis for bovine tissues: Conditions for maximising the number of positive cultures, in press, Copyright 2012, with permission from Elsevier.

The comparative performance of the single intradermal test and the single intradermal comparative tuberculin test in Irish cattle, using tuberculin PPD combinations of differing potencies

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The Veterinary Journal 190, e60-e65 (2011)

In national bovine tuberculosis (bTB) control programmes, testing is generally conducted using a single source of bovine purified protein derivative (PPD) tuberculin. Alternative tuberculin sources should be identified as part of a broad risk management strategy as problems of supply or quality cannot be discounted. This study was conducted to compare the impact of different potencies of a single bovine PPD tuberculin on the field performance of the single intradermal comparative tuberculin test (SICTT) and single intradermal test (SIT). Three trial potencies of bovine PPD tuberculin, as assayed in naturally infected bovines, namely, low (1192 IU/dose), normal (6,184 IU/dose) and high (12,554 IU/dose) were used. Three SICTTs (using) were conducted on 2,102 animals. Test results were compared based on reactor-status and changes in skin-thickness at the bovine tuberculin injection site. There was a significant difference in the

number of reactors detected using the high and low potency tuberculin. In the SICTT, high and low potency tuberculin detected 40% more and 50% fewer reactors, respectively, than normal potency tuberculin. Furthermore, use of the low potency tuberculin in the SICTT failed to detect 20% of 35 animals with visible lesions, and in the SIT 11% of the visible lesion animals would have been classified as negative. Tuberculin potency is critical to the performance of both the SICTT and SIT. Tuberculin of different potencies will affect reactor disclosure rates, confounding between-year or between-country comparisons. Independent checks of tuberculin potency are an important aspect of quality control in national bTB control programmes.

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The comparative performance of the single intradermal comparative tuberculin test in Irish cattle, using tuberculin PPD combinations from different manufacturers

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Ireland currently obtains its avian and bovine tuberculin purified protein derivatives (PPDs) from a single source. Because problems of supply or quality cannot be discounted, it is prudent that Ireland identify alternative supplier(s) as part of a broad risk management strategy. Therefore, the aim of this study was to compare the performance of a number of different tuberculin combinations (that is, pairings of bovine and avian PPD; with different manufacturers) in the single intradermal comparative tuberculin test (SICTT), as currently performed in Ireland. The study was randomised, controlled and double-blinded. A total of 2,172 cattle were used in the study. Each animal was tested using two SICTTs, the first based on the tuberculin combination in current use, and the second using one of six trial tuberculin combinations. Analyses were conducted to compare both reactor-status and skin increase. For each control/trial tuberculin combination, there was good agreement between the control and trial reactor-status. Differences in skin increases were mainly confined to animals categorised as either negative or severe inconclusive. However, the measured differences were minor, and unlikely to have a significant impact on the actual test outcome, either for individual animals or for herds. In conclusion, while further studies determining sensitivity and specificity in Ireland would have to be done in the event of a change in tuberculin PPD there should be minimal disruption of the national programme if alternative tuberculin PPDs meeting WHO, OIE and EU regulations were used. In this study, the precision of the guinea pig bio-assay to assess tuberculin potency was low and therefore Ireland should maintain its practice of periodically assessing potency in naturally infected cattle, even though this is not currently required under WHO, OIE or EU Regulations.

Reprinted from Veterinary Microbiology, 151, Good, M., Clegg, T.A., Murphy, F., More, S.J., The comparative performance of the single intradermal comparative tuberculin test in Irish cattle, using tuberculin PPD combinations from different manufacturers, 77-84, Copyright 2011, with permission from Elsevier.

Evaluation of the methodological quality of studies of the performance of diagnostic tests for bovine tuberculosis in cattle using QUADAS adapted for veterinary use

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A systematic review was conducted to identify studies that measured the performance of diagnostic tests for bTB in cattle. There has been little assessment of the methodological quality of these studies despite the importance of these tests in national surveillance and control schemes. Reference papers that appeared to have eligible data were reviewed using the QUADAS (Quality Assessment of Diagnostic Accuracy Studies) instrument adapted for veterinary use. During the study 190 references published between 1934 and 2009 were assessed using VETQUADAS by at least one of 18 reviewers. Of these, 107 were scored by 2 reviewers and 83 also had eligible estimates of sensitivity and or specificity for the systematic review. A similar pattern in the degree to which methodological criteria were met was observed across studies of different types of diagnostic tests. In references scored by two reviewers items measuring internal validity were assessed as having been met in 31-83% of studies. In only 31 and 45% of studies respectively did reviewers assess that the index test was interpreted without knowledge of the reference standard and the reference standard interpreted without knowledge of the index test. The review showed that there is considerable scope for improvement in the methodological quality of studies measuring performance of diagnostic tests for bTB.



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Systematic review to identify primary research estimating the performance of diagnostic tests for bovine tuberculosis in cattle

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The aim of this study was to conduct a systematic review to identify studies that have estimates of the sensitivity and specificity of diagnostic tests for bTB in cattle that could be used in a statistical meta-analysis of test performance. Detailed review was conducted by a working group of 18 reviewers. Comprehensive search criteria were developed and the process of review standardised. No limits were applied by year, language, region or type of diagnostic test in the initial search. 9,782 references were identified initially and abstracts, where available, were each reviewed by two reviewers. Entire references of those that passed through the initial review were randomly allocated to reviewers for detailed review if English language ($n=215$) and to native speaker reviewers if non-English language ($n=46$). An agreed range of data was extracted. There were 119 references with eligible performance estimates (published 1934-2009) for one or more of 14 different diagnostic tests. Studies varied in cattle population selection, reference standard and the thresholds and scales over which performance was measured. Methods for identifying studies that contain performance data for diagnostic tests for bTB are unspecific. Information provided in abstracts of studies of test performance needs to be standardised. Large scale studies of the performance of diagnostic tests for bTB in cattle are still needed.

Results from a meta-analysis of sensitivity and specificity of diagnostic tests for bovine tuberculosis in cattle

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A meta-analysis study was conducted using data extracted during a systematic review of references that reported the sensitivity (Se) and/or specificity (Sp) of diagnostic tests for bovine tuberculosis (bTB) in cattle. Performances were estimated, for 14 different diagnostic tests and modifications of the tests, using binary logistic regression models adjusting for relevant covariates. Parameters in the models were estimated using Monte Carlo Markov Chain with the category best representing test conditions in Great Britain and in Ireland as baseline. Estimates for the most common

tests used in GB for the interferon gamma blood test using bovine and bovine-avian PPD were 0.87 (95% Bayesian Credible Interval 0.72, 0.95) and 0.67 (95% BCI 0.49, 0.82), respectively, for Se and 0.97 (95% BCI 0.94, 0.98) and 0.98 (95% BCI 0.96, 0.99) for Sp and for the single intradermal comparative cervical tuberculin skin test using standard interpretation were 0.50 for Se (95% BCI 0.26, 0.78) and 1 (95% BCI 0.99, 1.00) for Sp.

Bovine tuberculosis: Effect of the tuberculin skin test on *in vitro* interferon gamma responses

Schiller, I.¹, Vordermeier, H.M.², Waters, W.R.³, Whelan, A.O.², Coad, M.², Gormley, E.⁴, Buddle, B.M.⁵, Palmer, M.³, Thacker, T.³, McNair, J.⁶, Welsh, M.⁶, Hewinson, R.G.², Oesch, B.⁷

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Veterinary Immunology and Immunopathology 136, 1–11 (2010)

Bovine tuberculosis (bTB) is a disease of zoonotic and economic importance. In many countries, control is based on test and slaughter policies and/or abattoir surveillance. For testing, cell mediated immune- (CMI-) based assays (i.e., tuberculin skin test (TST) supplemented by the interferon gamma (IFN-gamma) assay) are the primary surveillance and disease control tests for bTB. The combined use of the *in vivo* and *in vitro* CMI assays to increase overall sensitivity has raised the question of whether the IFN-gamma response is influenced by injection of purified protein derivatives (PPDs) for TST. Published data on the influence of the TST, applied as the caudal fold test (CFT) or the comparative cervical test (CCT), on the IFN-gamma assay are contradictory. Reviewing published data and including additional data, the following conclusions can be drawn: (1) in naturally infected cattle, PPD administration for the single or repeated short-interval CCT neither boosts nor depresses PPD-specific IFN-gamma production. Disparate results have been concluded from some studies using experimental infections, emphasizing the importance of confirming initial experimental-based findings with studies using cattle naturally infected with *Mycobacterium bovis*. (2) In cattle experimentally infected with *M. bovis*, PPD administration for CFT boosts PPD-specific IFN-gamma production for up to 7 days without any effect on test interpretation. Importantly, in naturally infected cattle, CFT-related boosting selectively increases the *in vitro* *M. bovis* PPD (PPD-B) response 3 days after CFT, resulting in an increased PPD-B response relative to the response to *Mycobacterium avium* PPD (PPD-A). In non-infected cattle, it cannot be excluded that the CFT induces a mild boost of the PPD-specific response, particularly in animals sensitized to environmental, non-tuberculous mycobacteria, thus decreasing the specificity of the IFN-gamma assay. (3) In general, there is a lack of data clearly characterizing the effect of TSTs on the IFN-gamma assay. Further studies are required to clearly describe the effects of both CFT and CCT in non-infected animals and in naturally infected cattle, especially in low reacting infected cattle.

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Development and evaluation of an enzyme-linked immunosorbent assay for use in the detection of bovine tuberculosis in cattle

Waters, W.R.¹, Buddle, B.M.², Vordermeier, H.M.³, Gormley, E.⁴, Palmer, M.V.¹, Thacker, T.C.¹, Bannantine, J.P.¹, Stabel, J.R.¹, Linscott, R.⁵, Martel, E.⁵, Milian, F.⁶, Foshaug, W.⁷, Lawrence, J.C.⁵

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Clinical and Vaccine Immunology 18, 1882-1888 (2011)

As a consequence of continued spillover of *Mycobacterium bovis* into cattle from wildlife reservoirs and increased globalization of cattle trade with associated transmission risks, new approaches such as vaccination and novel testing algorithms are seriously being considered by regulatory agencies for the control of bovine tuberculosis. Serologic tests offer opportunities for identification of *M. bovis* infected animals not afforded by current diagnostic techniques. The present study describes assay development and field assessment of a new commercial ELISA that detects antibody to *M. bovis* antigens MPB83 and MPB70 in infected cattle. Pertinent findings include: specific antibody responses were detected -90-100 days after experimental *M. bovis* challenge, minimal cross-reactive responses were elicited by infection/sensitization with non-tuberculous *Mycobacteria* spp., and the apparent sensitivity/specificity of the ELISA with naturally infected cattle was 63% / 98% respectively, with sensitivity improving as disease severity increased. The ELISA also detected infected animals missed by the routine tuberculin skin test and antibody was detectable in bulk tank milk samples from *M. bovis*-infected dairy herds. A high throughput ELISA could be adapted as a movement, border, or slaughter surveillance test, as well as a supplemental test to tuberculin skin testing.

Reprinted from Clinical and Vaccine Immunology, 18, Waters, W.R., Buddle, B.M., Vordermeier, H.M., Gormley, E., Palmer, M.V., Thacker, T.C., Bannantine, J.P., Stabel, J.R., Linscott, R., Martel, E., Milian, F., Foshaug, W., Lawrence, J.C., Development and evaluation of an enzyme-linked immunosorbent assay for use in the detection of bovine tuberculosis in cattle, 1882-1888, Copyright 2011, American Society for Microbiology.

Specificity of the tuberculin test in cattle herds adjacent to peat-land areas in Ireland

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In Ireland, where peat-lands occur on the margins of grassland, there is some concern that cattle may become sensitized to environmental mycobacteria, and result in false positive reaction to the tuberculin skin test, or a so called non-specific reactor (NSI). This is evidenced by the finding of low lesion rates in reactors from certain areas. To date, little work has been done to assess this issue of test specificity. This study describes the relationship between visible lesion rates at slaughterhouse among standard reactor animals and likelihood of exposure to environmental mycobacteria at pasture adjacent to peat land, within Co. Tipperary, over the period 2007-2009. The aim of this study is to model the likelihood of detecting a post mortem lesion at slaughter in standard reactors derived from herds grazing within 500m of peat land (exposed) compared to herds grazed more than 2km away from peat-land (unexposed). Landuse will be determined by reference to the Corine™ dataset, using ArcGIS software. We will analyze the data at the animal level. Data will be analysed using a multivariable logistic regression model with the presence of a visible lesion at slaughter as the positive outcome. Animals showing no visible lesion (NVL) will have negative outcomes. The exposure of interest will be proximity to peat-land. Other explanatory variables will include previous animal and herd-level bTB test readings, and other nationally available data.

Factory surveillance

Relative effectiveness of Irish factories in the surveillance of slaughtered cattle for visible lesions of tuberculosis, 2005-2007

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Irish Veterinary Journal (in press)

In Ireland, as part of the ongoing bovine tuberculosis (bTB) control/eradication scheme, every animal is examined at slaughter for its fitness for human consumption. The aim of this study was to determine the relative effectiveness of factories in submitting and subsequently in having these suspect lesions confirmed as bTB lesions during the years 2005-2007 in Ireland and to provide an update from previously published data for years 2003-2004. During 2005-2007 data were available on 4,401,813 cattle from attested herds, from which data for potential confounding factors was available for 3,344,057 animals slaughtered at export-licensed factories, of which there were 37 during that period. From these animals, 8,178 suspect lesions were submitted for laboratory confirmation. Lesions from 5,456 (66.7%) animals were tested as positive, 2,453 (30%) as negative and 269 (3.2%) were inconclusive for bTB. Logistic regression was used to determine adjusted submission and confirmation risks for each factory while controlling for confounding factors. Factory rankings based on adjusted and crude risks were similar. The average crude submission risk for all the factories was 25 lesions per 10,000 animals slaughtered, ranging from 0 to 52. The crude confirmation risk varied between 30.3% and 91.3%. Substantial variation between surveillance effectiveness in the 37 factories was found. Compared to previous years (2003-2004), there was an increased bTB lesion submission and confirmation risk. Continued monitoring of the effectiveness of slaughter surveillance in Ireland is recommended; emphasis should be placed on efforts to improve bTB surveillance in factories with lower rankings.

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Evaluation of a risk-based approach to meat inspection for bovine tuberculosis in Ireland

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The aim of this study was to consider the risk factors for the finding of suspect bovine tuberculosis (bTB) lesions at post mortem meat inspection in Ireland. Tuberculin testing results for bTB and laboratory submission of suspect post mortem bTB lesions, were analysed. The year 2009 was selected for analysis in which 1,515,217 bovines were slaughtered in Ireland. Multivariable logistic regression analysis of the data showed that the age of the animal was a good predictor of the presence of bTB lesions at meat inspection. In animals under the age of three, the suspect lesion identification rate is less than half the rate for animals aged three and above. The impact of a risk-based meat inspection system for bTB in Ireland, i.e. by a reduced meat inspection system for younger bovines, was considered.

Improving management of high risk herds

Introduced infection

Study of herds that introduced new animals during the course of a restriction for bovine tuberculosis

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During a movement restriction for bovine tuberculosis, a farmer may wish to buy new cattle in order to either replace animals lost as a result of bovine tuberculosis (bTB) or to enable routine management practices to continue 'as normal' (in spite of the bTB restrictions imposed). Permission to allow the restocking of restricted herds is currently an area of policy uncertainty, due to the unknown risks of the purchased animals. The objectives of this study are i) to determine whether restocking during restriction is associated with increased bTB risk, ii) to provide an overview of events associated with each restocked episode and to clarify the infection status of animals that are introduced during restrictions and iii) on the proviso that there is evidence of an increased bTB risk, to identify the practices relating to restocking that are the most risky and to determine whether the increased risk is associated with the source or the restocked herd.

Predictors of the first between-herd animal movement for cattle born in 2002 in Ireland

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Preventive Veterinary Medicine 97, 264-269 (2010)

Movement of animals between farms represents a potential risk of bovine tuberculosis (bTB) and other disease transmission. The objectives of this study were to identify and quantify risk factors associated with the first between-herd movement of animals (denoted as risk move). A random sample of 1 percent of Irish calf births registered for 2002 (20,182 animals) was selected. Descriptive and survival analysis on movement over the period 2002-2005 was performed. A total of 12,119 (60%) of animals experienced a risk move over the 4-year study period. Among those that moved, 57% did so within the first 12 months of age. For animals in dairy herds, an early peak in risk move events was observed within the first 12 weeks of age; whereas in animals from suckler herds, a later risk move peak was observed between 21 and 36 weeks of age. The survival models identified a number of risk factors: two that appeared most important in predicting a risk move were gender and enterprise type. Males had a hazard ratio of 2.6 times that of females. The hazards for enterprise type, varied over time, thus a time-varying covariate ($\text{ent_type} \times \ln(\text{time})$) was included in the Cox model. At 7 days of age, females in suckler herds were at 0.14 times the hazard of females in dairy herds for risk move, and over time, the hazards converged, equalised by day 140, and then diverged, so that by 4 years of age, females in suckler herds were at 4.64 times the hazard of females in dairy herds. Herds with a history of selling animals in previous years maintained that record during the study period with increased hazard of risk move. Enterprise type interacted with gender so that relative to females, males from dairy herds were at greater hazard of risk move than males from suckler herds. Hazard of risk move was also a function of $\ln(\text{herd area})$, so that each doubling of farm area was accompanied by a 30.6% decrease in the hazards. The main conclusion was that risk of movement related disease transmission also depends on the purpose of the movement whether for breeding or for beef finishing. While males were at greater

hazard of movement than females, they would have a shorter lifespan, thus limiting the opportunity for further transmission post-movement.

Reprinted from Preventive Veterinary Medicine, 97, White, P.W., Frankena, K., O’Keeffe, J., More, S.J., Martin, S.W., Predictors of the first between-herd animal movement for cattle born in 2002 in Ireland, 264-269, Copyright 2010, with permission from Elsevier.

Local TB persistence

Risk of bovine tuberculosis for cattle sold out from attested herds during year 2005 in Ireland: a descriptive analysis

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Bovine tuberculosis (bTB) is an infectious contagious disease caused by a bacterium, *Mycobacterium bovis* (*M. bovis*). The significance of bTB lies in trade implications and zoonotic potential. Although industrialized countries have significantly reduced the prevalence of *M. bovis* infection in both humans and cattle, bTB persists in some developed countries, including the Republic of Ireland. A retrospective cohort study was conducted to determine the risk of bTB among animals sold out from “clear” herds during the year 2005. Herds from which the animals were sold out were classified as “exposed” and “non-exposed” (our main risk factor) to bTB according to their previous bTB history during the year 2005. Our study sample was comprised of 338,960 animals, from which 124,360 were sold out from “exposed” herds and 214,600 animals were sold out from “non-exposed” herds. The overall risk of bTB during the 2-year period after the animals were sold out was 0.69%. The odds of testing positive to bTB was 1.94 (95% CI = 1.79-2.11, $p = <0.0001$) for animals sold out from “exposed” herds compared to animals sold out from “non-exposed” herds. Other confounding factors (age and gender) and bTB breakdown severity during 2005 were included in our analysis and had a significant association with the risk of bTB at the animal level in Ireland. This type of analysis should in the near future incorporate other risk factors in order to demonstrate quality assurance that can be supported, tracked and guaranteed.

A retrospective cohort study of the risk of TB among suckler calves whose dam tested positive to the single intradermal comparative tuberculin test (SICTT)

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The very close relationship of a suckling dam with her offspring and their contact via the suckling process and grooming raises the question of whether or not an infected dam could possibly transmit tuberculosis to her offspring via either the oral or respiratory route. The aim of this study is to assess the hypothesis that calves whose dams failed the Single Intradermal Comparative Tuberculin Test (SICTT) will have an increased risk of failing the test in the future compared to those whose dams were negative to the SICTT. A retrospective cohort study was designed. Data from all suckler herds which failed the SICTT test in Co. Clare in 2007 will be obtained from Animal Health Computer System (AHCS) operated by the Department of Agriculture, Food and the Marine (DAFM). A control sample of suckler cows negatives to the skin test and their respective calves will be also obtained.

Shorter-term risk of *Mycobacterium bovis* in Irish cattle following an inconclusive diagnosis to the single intradermal comparative tuberculin test

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Preventive Veterinary Medicine 102, 255-264 (2011)

In Ireland, new cases of bovine tuberculosis (bTB) are detected using both field and abattoir surveillance (More and Good, 2006). Field surveillance is conducted through annual testing of all cattle using the single intradermal comparative tuberculin test (SICTT). An animal may be deemed a 'standard inconclusive reactor' (SIR) to the SICTT if the bovine response is >2 mm and between 1 and 4 mm > the avian response. The herdowner then has three choices for the management of the SIR: option 1 is to have the animal retested after a minimum period of 42 days (an inconclusive reactor retest, IRR), option 2 is to slaughter the SIR and, provided the animal has no visible lesions, have a full herd test 42 days after the SIR leaves the herd, option 3 is to slaughter the SIR and have the lymph nodes examined using histology and/or culture for bTB. In the current study, we examine the bTB risk for SIRs both at slaughter prior to the IRR and at the IRR, and the future bTB risk of TIR animals (so-called 'transient SIRs'; SIR animals with a negative SICTT result at the subsequent IRR) that moved from the herd of disclosure within 6 months of the IRR. We also investigate factors associated with the future bTB status of SIRs at slaughter prior to the IRR and at the IRR. The study population included all SIRs identified in Ireland between 2005 and 2009 inclusive in a herd otherwise Officially TB free (OTF). Between 11.8% and 21.4% of SIRs slaughtered prior to the IRR were confirmed bTB positive at post mortem (using histology or culture if histology was not definitive), compared to 0.13–0.22% of SICTT –ve cohort animals. The post mortem bTB lesion rate of SIRs is lower than the lesion rate reported for reactor animals between 2005 and 2009 of between 34% and 39%, reflecting the doubtful infection status of these animals. Between 20.3% and 27.9% of herds were restricted at the IRR. The herd restriction rate amongst the national herd between 2005 and 2009 varied from 5.09% to 6.02%. TIRs that moved out of the disclosing herd within 6 months of the IRR were 12 times more likely to be bTB positive at the next test/slaughter compared to all animals in the national herd. The same increased risk did not apply to the SICTT –ve cohort animals that moved out of the same herds at the same time. Based on a range of measures, SIRs and TIRs are each at increased bTB risk into the future. Consequently, differential treatment of TIR animals would be justified.

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Longer-term risk of *Mycobacterium bovis* in Irish cattle following an inconclusive diagnosis to the single intradermal comparative tuberculin test

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Preventive Veterinary Medicine 100, 147-154 (2011)

In Ireland, new bovine tuberculosis (bTB) cases are detected using both field and abattoir surveillance. During field surveillance, an animal may be deemed a 'standard inconclusive reactor' (SIR) to the single intradermal comparative tuberculin test (SICTT) if the bovine response is >2mm, and from 1 to 4mm greater than the avian response. Little is known about the future infection risk posed by SIR animals that pass a subsequent retest, so-called 'transient SIR' (TIR) animals. The objective of this study was to critically evaluate the future bTB status of TIR animals, by examining the

future risk of bTB diagnosis over the 4 years following initial SIR diagnosis and clearance at the subsequent retest. The study included all TIRs that were identified as SIRs in 2005 in otherwise free herds at tests with no other reactors at that test and that were clear at the subsequent retest. The analysis was restricted to cows that were neither sold, other than direct to slaughter, nor exported from the herd during the follow up period (to the end of 2009). Five control cows were randomly selected from each study herd. A parametric survival model with shared frailties, to account for clustering within herds, was developed to model time from passing a retest to future bTB diagnosis. The final parametric survival model contained the variables: TIR status in 2005, inconclusive status during the follow-up period, location, herd restricted during the study, time since last restriction within the herd and age. The time ratio for the TIR status variable was significant ($p<0.001$) indicating that on average the time to diagnosis with bTB for TIRs was 78% shorter compared to the non-TIRs. The frailty term was significant ($p<0.001$) indicating that animals within some herds were more likely to become reactors compared to other herds. These results have important implications for national policy and future management of TIR animals. Further, private veterinary practitioners and their clients should be aware of the increased risk associated with TIRs.

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Effect of distance between a previously *Mycobacterium bovis*-infected herd and clear herds on the occurrence of bovine tuberculosis breakdowns in Ireland

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This study looks at herds surrounding a recently derestricted herd and seeks to answer the following question - what is the animal level bTB risk in the 4 calendar years following derestriction? We will conduct a retrospective cohort study, comparing herds that were (case herds) and were not (control herds) derestricted in 2005 (case herds). The study will focus on future animal test outcomes, up to end of 2008, for animals present within neighbouring herds. Herd-level explanatory variables (for case herds) include number of reactors, number of standard reactors, indices of length of restriction, and herd size at the 2005 completed episode. Among herds surrounding case herds, a matching number of control herds will be selected at random within county, as one from each of the following zones: i: From contiguous herds within 25m (direct neighbours) of a derestricted herd; ii: From contiguous herds within 26m-150m of a derestricted herd; iii: From contiguous herds within 151m – 1km of a derestricted herd. Each comparison herd will be randomly assigned an end date of restriction within 2005, stratified by month, to follow the same distribution as the case herds. For each case herd, and control herds, a list of animals present at the 2005 derestriction date will be reconstituted from CMMS records (the “study population”). Subsequent animal test outcomes for the study population will be compared up to the end of 2008 between animals from case vs. control herds.

Risk of bTB associated with animals moving out of derestricted herds

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This study seeks to answer the following question - for herds that were recently derestricted, what is the animal level bTB risk for animals sold to other herds compared to those remaining within the herds? We will conduct a retrospective cohort study. For herds derestricted in 2005 (case herds), a comparison of future animal test outcomes will be made with herds that remained unrestricted (control herds). Herd-level explanatory variables for case herds include number of reactors, number of standard reactors, indices of length of restriction, and herd size at the 2005 completed episode. For each case herd, a matching number of control herds will be selected from herds that remained unrestricted in 2005 and preceding years as follows: Group1: unrestricted during 2004 and 2005, Group2: unrestricted during 2003, 2004 and 2005 and Group3: unrestricted during 2002, 2003, 2004 and 2005. Each comparison herds will be randomly assigned an end date of restriction within 2005, allowing for seasonal variations to follow the same distribution as the case herds. For each case herd, and control herd, a list of animals present at the 2005 derestriction date will be reconstituted from CMMS records (the "study population"). Details of subsequent between-herd movements will be extracted from CMMS for the period up to end of 2008. Those moving out of their study herd to another herd will be classed as "movers", and the remainder classed as "non-movers". By means of a survival analysis, animal test outcomes for the study population will be compared over time between movers vs. non-movers reconstituted from case and control herds.

The temporal and spatial patterns of bovine tuberculosis in County Kilkenny cattle herds, 1998 to 2008

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The use of epidemiological methods is an intrinsic component of disease management and control. The objective of this study is to examine Bovine Tuberculosis (bTB) in County Kilkenny cattle herds from 1998 to 2008 inclusive and use the information to enhance the understanding of the epidemiology of the disease in the county. The study will examine the long term changes that have occurred over the decade (secular trends), and will investigate cyclical disease patterns and seasonal disease patterns. Spatial disease patterns may be visualised and analysed by using cartographic (mapping) or geographic information systems (GIS) methods.

Impact of the national full herd depopulation policy on the recurrence of bovine tuberculosis in Irish herds, 2003 to 2005

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Veterinary Record 169, 581 (2011)

This study evaluated the impact of the Irish herd bovine tuberculosis (bTB) depopulation policy (depopulation, disinfection, contiguous testing and local badger removal where implicated) on the recurrence of bTB infection, by comparing the future risk in restocked herds following depopulation for either bTB or bovine spongiform encephalopathy (BSE) during 2003 to 2005. Each herd was assigned a 'previous bTB risk', based on bTB history during the five years before depopulation. Future bTB risk was estimated, using a multivariable Cox proportional hazard model for time-to-breakdown for each study herd, to identify risk factors associated with bTB. Future bTB risk varied significantly by reason for depopulation and previous bTB risk. Herds depopulated for bTB (by definition, at high bTB risk) were not significantly different from BSE herds with no or a low previous bTB risk. BSE herds with a high previous bTB risk were found to be at significantly greater future bTB risk. Herd bTB depopulation measures, as currently applied in Ireland, are shown to be effective in enabling herds to attain and retain bTB freedom following restocking. Based on the data presented, and consistent with current knowledge of the bTB epidemiology, local badger removal contributes to efforts to limit recurrence of bTB in Ireland.

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The association between weather and bovine tuberculosis

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In a time when it is widely acknowledged that climate change is occurring globally, the issue of weather in relation to the epidemiology of bovine TB needs greater understanding. For the period 2005-2009, we examine the influence of weather variables on TB incidence in cattle herds together with well established risk factors in the area of west County Wicklow in the east of Ireland which until recently had a separate district veterinary office (DVO). Data for this study were obtained from three sources: Data from the national database of TB testing history (VetNet) maintained by the Department of Agriculture, Food and the Marine (DAFM), meteorological data from the Irish Meteorological Office and badger data from the Wildlife Unit of DAFM all for west County Wicklow. The data will be analysed using statistical tools related to generalized linear mixed models and time series. Initial results suggest that high rainfall is associated with increased bTB levels in cattle herds.

Estimation of the between-herd reproduction ratio for contiguous spread of bovine tuberculosis

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Bovine tuberculosis (bTB) is a recurring disease burden for the Irish cattle sector, with between 20,000-30,000 reactors being removed annually under the national herd testing program. While GIS techniques have demonstrated that clustering of bTB exists in cattle herds on a wider spatial scale, relatively little work has been published on the risk of bTB transmission among herds within 25m (i.e. directly contiguous) of one other. Neighbouring herds within 25m of a herd experiencing a new bTB episode (index herd) may come into contact with bTB infected cattle and could become infected with bTB as a result. Alternatively, both index and neighbouring herds could be exposed to a common infected wildlife source. The pattern of contiguous spread among herds within areas of differing badger density may provide some useful insights into the nature bTB clustering. The study population will consist of herds within Removal (lower badger density) and Reference (higher badger density) areas of the Four Area Project, comprising parts of Cork, Donegal, Kilkenny and Monaghan. By use of herd testing data for the period of proactive badger removal, between August 1997 and September 2002, we will identify herds experiencing one or more bTB episodes (index herds). Using LPIS data for 2001, we will determine contiguous herds within 25m of an index herd, and assess the previous bTB history of those (contiguous) herds as a potential source of bTB exposure for the index herd. Using this information we will develop a mathematical model to calculate the between-herd reproduction ratio (R_0) for a bTB episode among neighbouring herds at 25m. The key factor of interest as a determinant of R_0 will be treatment area (removal or reference).

How important is neighbourhood spread in the persistence of bovine tuberculosis in Irish cattle herds?

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A key feature of bovine tuberculosis (bTB) is local persistence of infection, characterised by recurrent episodes in one or more neighbouring herds. Local persistence could be attributed to: residual infection that persists in the index herd, despite testing following initial disclosure, neighbourhood or contiguous spread by cattle-to cattle transmission over farm boundaries, and/or infection of herds via a common wildlife source, e.g. the badger. A case-control study was conducted on the association between the occurrence of a bTB episode in cattle herds in the Republic of Ireland, and a number of possible risk factors; the bTB history of neighbouring herds within 1km, the herds own bTB history, herd size, enterprise type, number of farm fragments, and the purchasing of cattle. Neighbouring herds were divided into three zones based on their closest association with a study (own) herd as: zone 1 (0-25m), zone 2 (26-150m) and zone 3 (151-1000m). The characteristics of 3,909 herds that had a bTB episode in 2006 were compared with those of 98,872 herds that were bTB tested, and remained clear throughout 2006. The results highlight an association between bTB and an increased animal incidence within two subsets of neighbouring herds: (i) herds directly contiguous during the previous 2 years, and (ii) herds at a distance of > 25 metres in the previous year. Further studies will be necessary to determine to what extent the association at (i) may be confounded by the existence of a wildlife (badger) source.

Predictive models

Stochastic herd-level modelling of freedom from bTB infection for testing strategies applied in differing risk scenarios in Great Britain

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The performance of surveillance system components (including diagnostic tests and slaughterhouse inspection), which make up a bovine tuberculosis surveillance system were evaluated using a stochastic model. This first calculates the probability that a herd, with specific characteristics and bTB surveillance history, is free from infection. The sensitivity of further surveillance required to meet a 'target' probability of freedom from infection is then calculated and subsequent decisions on the selection of components, which offer adequate sensitivity, can be based on specificity (expected number of false positives), time to achieve freedom, and therefore cost. The model required several inputs including evidence from previous herd surveillance, the prior probability that the herd was infected (estimated using local prevalence of bTB), the distributions of animal-level sensitivity and specificity of diagnostic tests (derived from a meta-analysis of diagnostic test performance) and the risk of introduction of infection into the herd (derived from the observed incidence of bTB in GB risk scenarios). Where the probability of introduction of infection was high, it was difficult to achieve and maintain a high target probability of herd freedom from infection even with the most sensitive of components. Conversely, where introduction of infection was low, components with low sensitivity may still provide adequate probability of freedom from TB.

From explanation to prediction: developing a predictive model for recurrent bovine tuberculosis in Irish cattle herds

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Preventive Veterinary Medicine 94, 170-177 (2010)

There is a good understanding of factors associated with bovine tuberculosis (bTB) risk in Irish herds. As yet, however, this knowledge has not been incorporated into predictive models with the potential for improved, risk-based surveillance. The goal of the study was to enhance the national herd scoring system for bTB risk, thus leading to improved identification of cattle herds at high risk of recurrent bTB episodes. A retrospective cohort study was conducted to develop a statistical model predictive of recurrent bovine tuberculosis episodes in cattle herds in the Republic of Ireland. Herd-level disease history data for the previous 12 years, the previous 3 years, the previous episode, and the current-episode were used in survival analyses to determine the aspects of disease history that were predictive of a recurrent breakdown within 3 years of a cleared bTB episode. Relative to herds with 0-1 standard reactors in the current bTB episode, hazard ratios increased to 1.3 and 1.6 for herds with 2-5 and >5 standard reactors, respectively. Compared to herds with <30 animals, hazard ratios increased from 1.8 to 2.5 and then to 3.1 for herds with 30-79, 80-173, and >174 animals respectively. Relative to herds with <4 herd-level tests in the previous 3 years, herds with 4-5 and >5 tests had 1.1 and 1.4 times greater hazard of a bTB breakdown. Herds that did not have a bTB episode in the 5 years prior to their 2001 episode were 0.8 times less likely to breakdown in the next 3 years than herds that did. Herds breaking down in the spring or summer were 0.8 times less likely to suffer a recurrent breakdown than herds breaking down in autumn or winter (this was likely due to seasonality in testing regimes). The presence of a confirmed bTB lesion was not predictive of increased risk of recurrent bTB. Despite the availability of detailed disease history, the predictive ability of the model was poor. One explanation for this was that herds suffering a recurrence of bTB on their first test after clearing a bTB episode were different from herds that broke down later in the period at risk. Future research might need to include additional variables to identify which subsets of herd bTB episodes, if any, have identifiable features that are predictive of recurrent breakdowns.

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Supporting studies

Genetics, milk production

Evidence for genetic variance in resistance to tuberculosis in Great Britain and Irish Holstein-Friesian populations

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Here, we jointly summarise scientific evidence for genetic variation in resistance to infection with *Mycobacterium bovis*, the primary agent of bovine tuberculosis (TB), provided by two recent and separate studies of Holstein-Friesian dairy cow populations in Great Britain (GB) and Ireland. The studies quantified genetic variation within archived data from field and abattoir surveillance control programmes within each country. These data included results from the single intradermal comparative tuberculin test (SICTT), abattoir inspection for TB lesions and laboratory confirmation of disease status. Threshold animal models were used to estimate variance components for responsiveness to the SICTT and abattoir confirmed *M. bovis* infection. The link functions between the observed 0/1 scale and the liability scale were the complementary log-log in the GB, and logit link function in the Irish population. The estimated heritability of susceptibility to TB, as judged by responsiveness to the SICTT, was 0.16 (0.012) and 0.14 (0.025) in the GB and Irish populations, respectively. For abattoir or laboratory confirmation of infection, estimates were 0.18 (0.044) and 0.18 (0.041) from the GB and the Irish populations, respectively. Estimates were all significantly different from zero and indicate that exploitable variation exists among GB and Irish Holstein Friesian dairy cows for resistance to TB. Epidemiological analysis suggests that factors such as variation in exposure or imperfect sensitivity and specificity would have resulted in underestimation of the true values.

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Genetic correlations between measures of *Mycobacterium bovis* infection and economically important traits in Irish Holstein-Friesian dairy cows

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Mycobacterium bovis is the primary agent of tuberculosis (TB) in cattle. The failure of Ireland and some other countries to reach TB-free status indicates a need to investigate complementary control strategies. One such approach would be genetic selection for increased resistance to TB. Previous research has shown that considerable genetic variation exists for susceptibility to the measures of *M. bovis* infection, confirmed *M. bovis* infection, and *M. bovis*-purified protein derivative (PPD) responsiveness. The objective of this study was to estimate the genetic and phenotypic correlations

between economically important traits and these measures of *M. bovis* infection. A total of 20,148 and 17,178 cows with confirmed *M. bovis* infection and *M. bovis*-PPD responsiveness records, respectively, were available for inclusion in the analysis. First - to third-parity milk, fat, and protein yields, somatic cell count, calving interval, and survival, as well as first-parity body condition score records, were available on cows that calved between 1985 and 2007. Bivariate linear-linear and threshold-linear sire mixed models were used to estimate (co)variance components. The genetic correlations between economically important traits and the measures of *M. bovis* infection estimated from the linear-linear and threshold-linear sire models were similar. The genetic correlations between susceptibility to confirmed *M. bovis* infection and economically important traits investigated in this study were all close to zero. *Mycobacterium bovis*-PPD responsiveness was positively genetically correlated with fat production (0.39) and body condition score (0.36), and negatively correlated with somatic cell score (-0.34) and survival (-0.62). Hence, selection for increased survival may indirectly reduce susceptibility to *M. bovis* infection, whereas selection for reduced somatic cell count and increased fat production and body condition score may increase susceptibility to *M. bovis* infection.

Reprinted from Journal of Dairy Science 93, Bermingham, M.L., More, S.J., Good M., Cromie, A.R., Higgins, I.M., Berry, D.P., Genetic correlations between measures of Mycobacterium bovis infection and economically important traits in Irish Holstein-Friesian dairy cows, 5413-5422, Copyright (2010), with permission from the American Dairy Science Association.

Genotype by environment interaction of susceptibility to *Mycobacterium bovis* infection and economically important traits in Irish Holstein-Friesian cows across areas of low and high TB incidence

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Mycobacterium bovis is the primary agent of tuberculosis in cattle. There is considerable genetic variation for susceptibility to *M. bovis* infection, and both favorable and antagonistic genetic correlations exist between susceptibility to *M. bovis* infection and health, fertility and production traits in Irish Holstein-Friesian dairy cattle. The effectiveness of breeding program for increased resistance to TB and performance in other economically important traits may be hindered via genotype by environment interaction, whereby different genotypes respond differently to changes in the environment. The objective of this study was therefore to identify the existence and scope of genotype by environment interaction for susceptibility to *M. bovis* infection and other economically important traits of Holstein-Friesian dairy cattle across low and high TB incidence areas in Ireland. The single intradermal comparative tuberculin test (SICTT) was used as a measure of susceptibility of cows to *M. bovis* infection. A total of 19,892 cows with tuberculin test records between 2000 and 2008 were available for inclusion in the analysis. First to third parity milk, fat, and protein yield, somatic cell count, calving interval and survival, as well as first parity body condition score records, were available on cows calving between 1985 and 2007. Tuberculosis incidence in Ireland for the years 2000 to 2008 was mapped using Geographical Information Systems methodology. The data was split into two environments based on a SICTT reactor density threshold of 0.34 cattle per km² per year. Bivariate linear sire mixed models were used to estimate (co)variance components between traits across the high and low TB incidence environments. Homogeneous genetic variance was observed for variance response to the SICTT across the low and high incidence environments ($P > 0.05$). The genetic correlation between response to the SICTT in the low and high incidence environment was not significantly less than unity ($P > 0.05$); indicating that re-ranking of sires is limited. However, heterogeneous genetic correlations were estimated between three parity milk fat production and response to the SICTT across the high (0.30 - 0.35) and low (-0.10 - -0.02) incidence environments. Hence, selection for increased milk fat production will increase susceptibility to TB in high incidence areas. The absence of environmental sensitivity of response to the SICTT across environments indicates national level selection is feasible. The heterogeneous genetic correlation between milk fat production responses to the SICTT, however, needs to be considered in the design of breeding programs in order to accurately predict rate of genetic gain across TB incidence environments.

Genetics of animal health and disease in cattle

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Irish Veterinary Journal 64, 5 (2011)

There have been considerable recent advancements in animal breeding and genetics relevant to disease control in cattle, which can now be utilised as part of an overall programme for improved cattle health. This review summarises the contribution of genetic makeup to differences in resistance to many diseases affecting cattle. Significant genetic variation in susceptibility to disease does exist among cattle suggesting that genetic selection for improved resistance to disease will be fruitful. Deficiencies in accurately recorded data on individual animal susceptibility to disease are, however, currently hindering the inclusion of health and disease resistance traits in national breeding goals. Developments in 'omics' technologies, such as genomic selection, may help overcome some of the limitations of traditional breeding programmes and will be especially beneficial in breeding for lowly heritable disease traits that only manifest themselves following exposure to pathogens or environmental stressors in adulthood. However, access to large databases of phenotypes on health and disease will still be necessary. This review clearly shows that genetics make a significant contribution to the overall health and resistance to disease in cattle. Therefore, breeding programmes for improved animal health and disease resistance should be seen as an integral part of any overall national disease control strategy.

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Bovine tuberculosis and milk production in infected dairy herds in Ireland

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Preventive Veterinary Medicine 93, 153-161 (2010)

This study describes the relationship between bovine tuberculosis (TB) and milk yield in TB-infected dairy herds in Ireland. The study had two objectives: to determine whether cows detected as TB reactors (and thus subject to immediate slaughter) were likely to be the higher milk-producing cows, and to determine whether subclinical TB infection was associated with reduced milk production at or around the time of disclosure (detection). All Irish dairy herds restricted from trading between the 1st June 2004 and the 31st May 2005 as a result of two or more TB reactors by the Single Intradermal Comparative Tuberculin Test (SICTT) were considered for study. The data consisted of 419 herds. Data were collected on all TB reactors and a random sample of 5 non-reactor cows in these herds: a data set of 4,340 cows (2,342 TB reactors and 1,998 non-reactors). Previous milk data for the cows were taken into consideration and thus all lactations on a cow were analysed together with the years of lactations. There was an inherent hierarchical structure in the data, with lactations nested within cows and cows within herds and thus a linear mixed model with two random effects was used to describe the data. The results of this study showed that for all lactations and years under investigation, milk yield was significantly lower for TB reactor cows, with differences ranging from 120kg (2003, lactation 3) to 573kg (2001, lactation 1), when compared to the non-reactor cows.

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Bovine tuberculosis and udder health in Irish dairy herds

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The Veterinary Journal (in press)

The association between bovine tuberculosis (TB) infection status based on results from the Single Intradermal Comparative Tuberculin Test (SICTT) and milk production has been described in dairy cows in TB-infected herds in Ireland. The biological basis was uncertain, but could be related to increased TB susceptibility among lower producing dairy cows. In this study, the relationship between somatic cell count (as an objective measure of udder health) and SICTT reactivity (as a proxy for TB infection status) was investigated. Somatic cell counts of TB infected cows, both during and prior to the lactation of diagnosis of TB infection, were examined and compared to non-infected cows. All Irish dairy herds restricted from trading between June 2004 and May 2005 as a result of two or more TB reactors (test positive) to the SICTT were considered for study. Data were collected on 4,340 cows from 419 herds. Previous lactation data for the cows were taken into consideration and all lactations of a cow were analysed together with the years of lactations. There was an inherent hierarchical structure in the data, with lactations nested within cows and cows within herds and so a linear mixed model with two random effects was used to describe the data. Milk production (305-day milk yield) was also included in the model as a fixed effect. The results of the study showed that for all lactations and years under investigation, somatic cell counts for SICTT reactor cows when compared to the non-reactor cows were not significantly different. In this study population, TB infection status was not associated with udder health.

Reprinted from The Veterinary Journal, Boland, F., Kelly, G.E., Good, M., More, S.J., Bovine tuberculosis and udder health in Irish dairy herds, in press, Copyright 2012, with permission from Elsevier.

Evidence of genetic resistance of cattle to infection with *Mycobacterium bovis*

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Journal of Dairy Science 93, 1234-1242 (2010)

Anecdotal evidence points to genetic variation in resistance of cattle to infection with *Mycobacterium bovis*, the causative agent of bovine tuberculosis (bTB), and published experimental evidence in deer and cattle suggests significant genetic variation in resistance and reactivity to diagnostic tests. However, such genetic variation has not been properly quantified in the United Kingdom dairy cattle population; it is possible that it exists and may be a factor influencing the occurrence of bTB. Using models based on the outcome of the process of diagnosis (ultimate fate models) and on the outcome of a single stage of diagnosis (continuation ratio models, herd test-date models), this study shows that there is heritable variation in individual cow susceptibility to bTB, and that selection for milk yield is unlikely to have contributed to the current epidemic. Results demonstrate that genetics could play an important role in controlling bTB by reducing both the incidence and the severity of herd breakdowns.

Reprinted from Journal of Dairy Science, 93, Brotherstone, S., White, I.M.S., Coffey, M., Downs, S.H., Mitchell, A.P., Clifton-Hadley, R.S., More, S.J., Good, M., Woolliams, J.A., Evidence of genetic resistance of cattle to infection with Mycobacterium bovis, 1234-1242, Copyright 2010, with permission from the American Dairy Science Association.

Genome-wide transcriptional profiling of peripheral blood leukocytes from cattle infected with *Mycobacterium bovis* reveals suppression of host immune genes

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BMC Genomics 12, 611 (2011)

Mycobacterium bovis is the causative agent of bovine tuberculosis (bTB), a pathological infection with significant economic impact. Recent studies have highlighted the role of functional genomics to better understand the molecular mechanisms governing the host immune response to *M. bovis* infection. Furthermore, these studies may enable the identification of novel transcriptional markers of bTB that can augment current diagnostic tests and surveillance programmes. In the present study, we have analysed the transcriptome of peripheral blood leukocytes (PBL) from eight *M. bovis*-infected and eight control non-infected age-matched and sex-matched Holstein-Friesian cattle using the Affymetrix® GeneChip® Bovine Genome Array with 24,072 gene probe sets representing more than 23,000 gene transcripts. Control and infected animals had similar mean white blood cell counts. However, the mean number of lymphocytes was significantly increased in the infected group relative to the control group ($P = 0.001$), while the mean number of monocytes was significantly decreased in the bTB group ($P = 0.002$). Hierarchical clustering analysis using gene expression data from all 5,388 detectable mRNA transcripts unambiguously partitioned the animals according to their disease status. In total, 2,960 gene transcripts were differentially expressed (DE) between the infected and control animal groups (adjusted P -value threshold ≤ 0.05); with the number of gene transcripts showing decreased relative expression (1,563) exceeding those displaying increased relative expression (1,397). Systems analysis using the Ingenuity® Systems Pathway Analysis (IPA) Knowledge Base revealed an over-representation of DE genes involved in the immune response functional category. More specifically, 64.5% of genes in the affects immune response subcategory displayed decreased relative expression levels in the infected animals compared to the control group. This study demonstrates that genome-wide transcriptional profiling of PBL can distinguish active *M. bovis*-infected animals from control non-infected animals. Furthermore, the results obtained support previous investigations demonstrating that mycobacterial infection is associated with host transcriptional suppression. These data support the use of transcriptomic technologies to enable the identification of robust, reliable transcriptional markers of active *M. bovis* infection.

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Impact of delayed processing of bovine peripheral blood on differential gene expression

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Veterinary Immunology and Immunopathology (in press)

RT-qPCR can be used to accurately determine expression levels of genes following RNA extraction from tissue samples. If blood is the source of total RNA, it is often desirable to process the samples immediately following collection because delays in processing for RNA extraction may influence mRNA expression estimates obtained from RT-qPCR analyses.

However, this may not be feasible if the site of blood collection is distant from the processing laboratory. In the present study, the effects of delays in the processing of blood samples on mRNA expression data was investigated using a panel of 23 functionally diverse genes from five different gene ontology (GO) categories in peripheral blood sampled from ten age-matched healthy cattle. Venous blood was collected in Tempus™ Blood RNA tubes, which contain reagents that lyse blood cells immediately and stabilise the RNA signature (*Tb*). Blood was also collected in conventional lithium heparin collection tubes, and stored at ambient temperature for *T*₄, *T*₆ and *T*₈ h, prior to total RNA extraction. The mRNA expression profiles of these 23 genes were determined by RT-qPCR and compared across the time course. Thirteen genes showed significant up- or down-fold changes in mRNA expression over the 8 h time course. Among the GO categories, genes in the Immune Response category showed the most differential expression. These results also demonstrated that the changes in mRNA expression for the *IFNG* gene, which encodes the cytokine IFN-γ, did not correspond to IFN-γ protein levels estimated using ELISA.

Reprinted from Veterinary Immunology and Immunopathology 145, Sheridan, M.P., Browne, J.A., MacHugh, D.E., Costello, E., Gormley, E., Impact of delayed processing of bovine peripheral blood on differential gene expression, 199-205, Copyright 2012, with permission from Elsevier.

Transcriptional profiling of immune genes in bovine monocyte-derived macrophages exposed to bacterial antigens

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Veterinary Immunology and Immunopathology 140, 130-139 (2011)

The involvement of Toll-like receptors (TLRs) and other immune signalling genes during challenge of bovine macrophages with bacterial products derived from disease-causing bacteria in cattle was investigated. An *in vitro* cell culture model of bovine monocyte derived macrophages (MDM) was established and these cells were exposed to purified protein derivative (PPD-b) derived from *Mycobacterium bovis* and to lipopolysaccharide (LPS) derived from *Escherichia coli*. Following 24h incubation, total RNA was extracted and expression of immune related genes was determined by real time quantitative reverse transcription PCR (qRT-PCR). Expression of a selection of genes spanning the TLR-2 and TLR-4 pathways, from the initial activation of the receptors to the production of pro-inflammatory cytokines and chemokines was determined. Results from repeat experiments using MDM from seven different age-matched dairy cattle showed that PPD-b treatment caused significant up-regulation of the *TLR2* and *TLR4* genes and the expression profile of TLR adaptor molecules suggested that this signalling is MYD88-dependent. Conversely, LPS caused significant up-regulation of *TLR4* via a MYD88-independent signalling pathway. Significant up-regulation of genes involved with NF-κB signalling was also detected in PPD-b- and LPS-treated samples accompanied by the expression of pro-inflammatory cytokine (*TNF*, *IL1B*, *IL6*) and chemokine genes (*IL8*, *CCL5*, *CCL3*). Overall, LPS challenge resulted in a more marked up-regulation of immune-related genes. Furthermore, the magnitude fold-change difference in gene expression suggests, at least in part, that bovine macrophages produce IFN-γ as a result of LPS challenge.

Reprinted from Veterinary Immunology and Immunopathology, 140, Taraktoglou, M., Szalabska, U., Magee, D.A., Browne, J.A., Sweeney, T., Gormley, E., MacHugh, D.E., Transcriptional profiling of immune genes in bovine monocyte-derived macrophages exposed to bacterial antigens, 130-139, Copyright 2011, with permission from Elsevier.

Tuberculosis in other farmed livestock species

Granuloma encapsulation is a key factor for containing tuberculosis infection in minipigs

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PLoS One 5, e10030 (2010)

A transthoracic infection involving a low dose of *Mycobacterium tuberculosis* has been used to establish a new model of infection in minipigs. The 20-week monitoring period showed a marked Th1 response and poor humoral response for the whole infection. A detailed histopathological analysis was performed after slicing the formalin-fixed whole lungs of each animal. All lesions were recorded and classified according to their microscopic aspect, their relationship with the intralobular connective network and their degree of maturity in order to obtain a dissemination ratio (DR) between recent and old lesions. CFU counts and evolution of the DR with time showed that the proposed model correlated with a contained infection, decreasing from week 9 onwards. These findings suggest that the infection induces an initial Th1 response, which is followed by local fibrosis and encapsulation of the granulomas, thereby decreasing the onset of new lesions. Two therapeutic strategies were applied in order to understand how they could influence the model. Thus, chemotherapy with isoniazid alone helped to decrease the total number of lesions, despite the increase in DR after week 9, with similar kinetics to those of the control group, whereas addition of a therapeutic *M. tuberculosis* fragment-based vaccine after chemotherapy increased the Th1 and humoral responses, as well as the number of lesions, but decreased the DR. By providing a local pulmonary structure similar to that in humans, the mini-pig model highlights new aspects that could be key to a better understanding tuberculosis infection control in humans.

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Tuberculosis in goats on a farm in Ireland: epidemiological investigation and control

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Veterinary Record 168, 485 (2011)

This paper describes an outbreak of tuberculosis (TB) caused by *Mycobacterium bovis* in a dairy goat herd on a farm in Ireland, where 66.3 per cent of the herd tested positive to the single intradermal comparative tuberculin test (SICTT) at initial detection. An epidemiological investigation was conducted to determine the origin of the outbreak, considering issues such as animal movements and herd management practices. Infection was introduced with a consignment of goats, as determined by the variable number tandem repeat profile. Infection was eradicated using a test and cull programme involving the SICTT, the interferon- γ assay and a multiplex immunoassay (Enferplex TB).

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Application of the Enfer Chemiluminescent Multiplex ELISA System for the detection of *Mycobacterium bovis* infection in goats

Shuralev, E.¹, Quinn, P.¹, Doyle, M.², Duignan, A.³, Kwok, H.F.⁴, Bezoz, J.⁵, Olwill, S.A.⁴, Gormley, E.², Aranaz, A.⁵, Good, M.³, Davis, W.C.⁶, Clarke, J.¹, Whelan, C.¹

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Veterinary Microbiology (in press)

A study was conducted to optimise a multiplex serological immunoassay for use in identification of goats infected with *Mycobacterium bovis*. To assess assay specificity, 31 goats with a history of being free from *M. bovis* infection were used. To determine assay sensitivity, 180 Single Intradermal Comparative Tuberculin test (SICTT) positive goats were recruited. Additionally, 286 SICTT negative goats classed as potentially exposed animals present in the same positive herds were also included in the study. The results of the assay demonstrated a specificity of 100%. The multiplex assay detected 57/60 SICTT (95.0%) positive animals in one *M. bovis* infected herd and 120/120 (100%) in a second herd. In a separate experiment, 28 *M. caprae* culture confirmed infected goats from Spain were assayed, of which 24 (85.7%) were found positive in the test. The results show that inclusion of an antibody based assay can improve the ability to identify *M. bovis* and *M. caprae* infected goats. With further development and validation the multiplex assay may prove to be a useful tool for control of *M. bovis* and *M. caprae* infection in goats.

Reprinted from Veterinary Microbiology 154, Shuralev, E., Quinn, P., Doyle, M., Duignan, A., Kwok, H.F., Bezoz, J., Olwill, S.A., Gormley, E., Aranaz, A., Good, M., Davis, W.C., Clarke, J., Whelan, C., Application of the Enfer Chemiluminescent Multiplex ELISA System for the detection of Mycobacterium bovis infection in goats, 292-297, Copyright 2012, with permission from Elsevier.

Improved understanding of ecology and TB epidemiology

Badger ecology

Can Eurasian badger (*Meles meles*) numbers be predicted from sett attributes and capture history? An application and evaluation of multivariable modelling

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Predicting badger numbers from sett characteristics and capture histories is of considerable applied importance. This ability would be useful in generating probable badger densities for disease and strategic models of bovine tuberculosis (bTB) control. Modelling is of interest to elucidate the factors that may impact on badger capture at local scales. Furthermore, badger management and vaccination programs would benefit by increasing the probability of efficiently capturing the target badger populations. Within this context, it will be investigated whether badger capture numbers can be predicted from field signs and previous capture histories. The relative benefits of different modelling approaches will also be explored (GLM, GEE, Zero-inflated with Poisson or Negative Binomial distributions). The different modelling techniques will be compared in terms of mean predicted error and coverage.

Impact of culling on relative abundance of the Eurasian badger (*Meles meles*) in three counties in Ireland

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The Eurasian badger (*Meles meles*) has been implicated in the epidemiology of bovine tuberculosis (bTB) in cattle populations in the Republic of Ireland. Badger populations have been subject to a regulated culling regime in areas with chronic histories of bTB cattle herd breakdowns. Removal data collected during this regime from 2004 to 2010 will be used to model the impact of culling on populations in areas under capture. Additionally, changes in field signs of badger activity will be used as an index of abundance to verify the outcomes of the removal models. The removal intensities will also be investigated and compared with previous experimental culls. These models will elucidate trends in badger population density over time in response to the culling regime.

The ecology of the Eurasian badger (*Meles meles*) in Ireland: a review

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There has been extensive research on the ecology of the Eurasian badger in Ireland. Despite much of the recent literature focusing on disease (bovine tuberculosis) dynamics relating to badgers, a great deal of insight into the autecology of the species in Ireland has emerged. A study will be undertaken to review all relevant and available studies relating to Irish badger populations. Particular effort will be made to investigate ‘grey literature’ (non-peer

reviewed material, including national and departmental research reports and theses) - often ignored work that may contain valuable observations. The study will also compare the differences and similarities of badger populations both within, and outside of Ireland.

Diet of the European badger (*Meles meles*) in the Republic of Ireland: A comparison of results from an analysis of stomach contents and rectal faeces

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Mammalian Biology 76, 470-475 (2011)

The diets of mammals have been investigated primarily through the analysis of faecal samples. In our study we analysed both stomach contents, and rectal faeces from European badgers. This approach enabled a direct comparison of the information derived from these two sources. The dietary components found from each source were the same. However, it was found that, compared to stomach contents, the contribution to the diet, by volume, of plant litter, earthworms, Tipulid larvae and adult Carabid beetles were significantly overestimated by faecal analysis, while those of Noctuid larvae and Carabid beetle larvae were significantly underestimated. The analysis of stomach contents showed clear evidence of seasonality in the consumption of earthworms, Carabid beetle larvae, Tipulid larvae and Noctuid larvae. This seasonality was not as evident when the diet was inferred by the analysis of faeces. We propose that an analysis of stomach contents rather than of faeces, more accurately reflects the relative proportions of ingested food types, and the seasonality of the diet.

Reprinted from Mammalian Biology, 76, Cleary, G.P., Corner, L.A.L., O'Keeffe, J., Marples, N.M., Diet of the European badger (Meles meles) in the Republic of Ireland: A comparison of results from an analysis of stomach contents and rectal faeces, 470-475, Copyright Deutsche Gesellschaft für Säugetierkunde 2011, with permission from Elsevier.

Analysis of movement patterns of satellite-tracked badgers in Co. Wicklow

Delaney, J.¹, Povey, C.¹, Fay, B.¹, Donnelly, S.¹, Mullen, E.², MacWhite, T.³, Maher, P.³, Gormley, E.⁴, Good, M.³, Kelly, D.J.¹, Marples, N.M.¹

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We have used home range data from satellite-tracked badgers to plot 'Minimum Convex Polygon' (MCP) home ranges for each badger in each month and have looked at the trends in average MCP values throughout the year. The results have shown that home ranges for the majority of individuals were greatest in summer and smallest in winter. This provides support for our current knowledge of the activity of badgers, as we know that they are relatively inactive in winter and much more active in summer. However, some individuals appear to use almost their whole summer range throughout the winter season, suggesting that there is greater variation in winter behaviour than has been found previously. We are also investigating whether age or sex have any noticeable effects on home range size. To do this we are using kernel estimates (generally considered to be the best assessment of home ranges) as well as MCPs, in order to get a more accurate picture of their behaviour. The satellite-tracking information on badger movements is also being used to study the effects of temperature and precipitation on the home ranges by comparing the data generated during a mild winter (2011-12) and a relatively cold winter (2010-2011). We are modelling the variation of MCP and kernel estimates of the badgers with age, sex, county, rainfall and temperature data. From these models we hope to identify the factors that best explain the variance in the dataset. Another study is looking at the frequency of badger incur-

sions into or near to farm buildings, and whether they actively avoid such incursions. We are also examining the preferences of badgers for foraging in different vegetation types. The results of this work will provide fundamental information on the nature of badgers' interactions with farms and may have implications for the transmission rates of tuberculosis from badgers to cattle.

The effect of badger removal on road casualty mammals

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Irish Naturalists' Journal 31, 118-122 (2010)

Records of a five year survey of mammalian road casualties in two different parts (removal and reference areas) of the Co. Cork portion of the Four Area Badger Project are reported. There were more hedgehog, domestic cat and fox casualties in the removal area, but only the first two species differed to a statistically significant extent from the reference area. How such studies might be improved and the possible implications for epidemiology and biodiversity are discussed.

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Badger road casualties in rural areas of the Republic of Ireland

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Road casualties are a cause of mortality for badgers. This project will look at such casualties in relation to a number of previously estimated populations in rural areas in the Republic of Ireland.

The badgers (*Meles meles* (L.)) of Little Island, Co. Waterford

Sleeman, D.P.¹, Partridge, T.², O'Boyle, I.², Gormley, E.³, Toolan, D.⁴

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Irish Naturalists' Journal 31, 94-99 (2010)

A high density island population of badgers is described. The methods used in the study to capture, anaesthetize and identify badgers, as well as to map territories, are detailed. The island had eight main setts and six territories. One territory had three main setts, two of which had small sub-territories. Badgers captured in the initial study, and those found dead from 1998-2007, are detailed. The finding of badgers without tuberculosis which were unwell (emaciated, with heavy ectoparasite infestations), in buildings is recorded. The implications for badger research and disease management are discussed.

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TB epidemiology

Estimating the extent of spatial association of *Mycobacterium bovis* infection in badgers in Ireland

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Epidemiology and Infection 138, 270-279 (2010)

Mycobacterium bovis infects the wildlife species badgers *Meles meles* who are linked with the spread of the associated disease tuberculosis (TB) in cattle. Control of livestock infections depends in part on the spatial and social structure of the wildlife host. Here we describe spatial association of *M. bovis* infection in a badger population using data from the first year of the Four Area Project in Ireland. Using second-order intensity functions, we show there is strong evidence of clustering of TB cases in each the four areas, i.e. a global tendency for infected cases to occur near other infected cases. Using estimated intensity functions, we identify locations where particular strains of TB cluster. Generalized linear geostatistical models are used to assess the practical range at which spatial correlation occurs and is found to exceed 6 in all areas. The study is of relevance concerning the scale of localized badger culling in the control of the disease in cattle.

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Spatial clustering of TB-infected cattle herds prior to and following proactive badger removal

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Epidemiology and Infection 139, 1220-1229 (2011)

Bovine tuberculosis (TB) is primarily a disease of cattle. In both Ireland and the UK, badgers (*Meles meles*) are an important wildlife reservoir of infection. This paper examined the hypothesis that TB is spatially correlated in cattle herds, established the range of correlation and the effect, if any, of proactive badger removal on this. We also re-analysed data from the Four Area Project in Ireland, a large-scale intervention study aimed at assessing the effect of proactive badger culling on bovine TB incidence in cattle herds, taking possible spatial correlation into account. We established that infected herds are spatially correlated (the scale of spatial correlation is presented), but at a scale that varies with time and in different areas. Spatial correlation persists following proactive badger removal.

Reprinted from Epidemiology and Infection, 139, Kelly, G.E., More, S.J., Spatial clustering of TB-infected cattle herds prior to and following proactive badger removal, 1220-1229, Copyright 2011, with permission from Cambridge University Press.

Tuberculosis in cattle herds are sentinels for *Mycobacterium bovis* infection in European badgers (*Meles meles*): the Irish Greenfield Study

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Veterinary Microbiology 151, 120-125 (2011)

In Ireland, badgers are removed in response to tuberculosis (TB) breakdowns in cattle herds (focal culling). Prevalence studies, conducted using a detailed post mortem and bacteriological examination, showed that 36-50% of badgers were infected with *Mycobacterium bovis*. Focal culling forms part of the medium term national strategy for the control of bovine TB in cattle and is based on the premise that badgers in areas with herd breakdowns have a higher prevalence of infection than the badger population at large. However, the hypothesis that cattle can be used as sentinels for infection in the badger population has never been formally tested. In this study we tested the hypothesis by determining the infection prevalence in badgers in areas where there had been historically, a consistently low prevalence of infection in cattle. Low cattle TB prevalence areas were defined as those herds with ≤ 2 standard reactors in the annual round of skin testing over the preceding 5 years (Greenfield sites). Using GIS, and adjusting for variation in land use, previous culling and cattle density, 198 Greenfield sites were identified and surveyed, and 138 areas with badger setts or signs of badger activity were identified. A single badger was removed from 87 sites and all were examined using detailed post mortem and bacteriological procedures. A prevalence of *M. bovis* infection of 14.9% was found in the Greenfield site badgers. This prevalence was significantly lower ($P < 0.001$) than in badgers removed during focal culling (36.6%). The results validate the use of cattle as sentinels for TB in badgers and support the medium term national strategy for the control of bovine TB. The geographic variation in *M. bovis* infection prevalence in the Irish badger populations will be used when devising strategies for the incorporation of badger vaccination into the long term bovine TB control programme.

Reprinted from Veterinary Microbiology, 151, Murphy, D., Gormley, E., Collins, D.M., McGrath, G., Sovsic, E., Costello, E., Corner, L.A.L., Tuberculosis in cattle herds are sentinels for Mycobacterium bovis infection in European badgers (Meles meles): the Irish Greenfield Study, 120-125, Copyright 2011, with permission from Elsevier.

Mycobacterial infections in multiple species: Implications for diagnosis and control

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The Veterinary Journal (in press)

This paper discusses the significance of the ever-increasing numbers of different pathogenic mycobacterial species affecting both domestic and wild animals. Through advances in diagnostics and increased epidemiological investigations, it should be possible to define the reservoir status of each animal host and to establish the significance in terms of infection risk.

Adapted from The Veterinary Journal 191, Corner, L.A.L., Gormley, E., Mycobacterial infections in multiple species: Implications for diagnosis and control, 141-142, Copyright 2012, with permission from Elsevier.

Mycobacterium bovis infection in the Eurasian badger (*Meles meles*): the disease, pathogenesis, epidemiology and control

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Journal of Comparative Pathology 144, 1-24 (2011)

Eurasian badgers (*Meles meles*) are an important wildlife reservoir of tuberculosis (*Mycobacterium bovis*) infection in Ireland and the United Kingdom. As part of national programmes to control tuberculosis in livestock, considerable effort has been devoted to studying the disease in badgers and this has led to a rapid increase in our knowledge of tuberculosis in this host. Tuberculosis in badgers is a chronic infection and in a naturally-infected population the severity of disease can vary widely, from latent infection (infection without clinical signs and no visible lesions) to severe disease with generalized pathology. The high prevalence of pulmonary infection strongly supports the lungs as the principal site of primary infection and that inhalation of infectious aerosol particles is the principal mode of transmission. However, other routes, including transmission via infected bite wounds, are known to occur. The ante mortem diagnosis of infection is difficult to achieve, as clinical examination and immunological and bacteriological examination of clinical samples are insensitive diagnostic procedures. Because infection in the majority of badgers is latent, the gross post mortem diagnosis is also insensitive. A definitive diagnosis can only be made by the isolation of *M. bovis*. However, to gain a high level of sensitivity in the bacteriological examination, a large number of tissues from each badger must be cultured and sensitive culture methods employed. The transmission and maintenance of *M. bovis* in badger populations are complex processes where many factors influence within-population prevalence and rates of transmission. Badger social structures and the longevity of infected animals make them an ideal maintenance host for *M. bovis* infection. Badgers are directly implicated in the transmission of infection to cattle and the inability to eradicate the disease from cattle is, in part, a consequence of the interactions between the two species. A detailed understanding and knowledge of the epidemiology and pathogenesis of the disease are recognized as fundamental for devising new strategies to control infection with a view to limiting interspecies transmission. Vaccination, in spite of formidable challenges, is seen as the best long-term strategy option and studies with captive badgers have shown that vaccination with *M. bovis* Bacillus Calmette-Guérin (BCG) induces protection when delivered by a variety of routes. Continued research is required to develop effective technologies to control the disease both in badgers and cattle. A combination of strategies, which employ the optimal use and targeting of resources, is likely to make a significant contribution towards eradication of the disease.

Adapted from Journal of Comparative Pathology, 144, Corner, L.A.L., Murphy, D., Gormley, E., Mycobacterium bovis infection in the Eurasian badger (Meles meles): the disease, pathogenesis, epidemiology and control, 1-24, Copyright 2011, with permission from Elsevier.

The prevalence and distribution of *Mycobacterium bovis* infection in European badgers (*Meles meles*) as determined by enhanced post mortem examination and bacteriological culture

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Research in Veterinary Science 88, 1-5 (2010)

The accurate diagnosis of *Mycobacterium bovis* infection in badgers is key to understanding the epidemiology of tuberculosis in this species and has significant implications for devising strategies to limit spread of the disease. In this study, badgers ($n=215$) in the Republic of Ireland were examined post mortem and tissues were collected from a range of anatomical locations and pooled into groups for bacterial culture of *M. bovis*. By assessing confirmed gross visible lesions

(VL) alone, infection was detected in 12.1% of badgers. However, by including the results of all culture positive pooled samples, the overall infection prevalence increased significantly to 36.3%. Two-thirds (66.7%) of infected animals had no visible lesions (NVL). While the thoracic cavity (lungs and pulmonary lymph nodes) was found to be the most common site of infection, in a proportion of animals infection was absent from the lungs and draining lymph nodes and was confined to the lymph nodes of the carcase or the head. This may indicate an early extrapulmonary dissemination of infection or alternatively, in the case of the head lymph nodes, a secondary pathogenic pathway involving the lymphoid tissues of the upper respiratory tract (URT).

Reprinted from Research in Veterinary Science, 88, Murphy, D., Gormley, E., Costello, E., O'Meara, D., Corner, L.A.L., The prevalence and distribution of Mycobacterium bovis infection in European badgers (Meles meles) as determined by enhanced post mortem examination and bacteriological culture, 1-5, Copyright 2010, with permission from Elsevier.

Infection control strategies

Badger removal

A comparison of the effectiveness of badger removal programs: Wildlife Administration Unit vs. pre-2004 licenced capture

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In response to severe outbreaks of tuberculosis in cattle, an epidemiological investigation is carried out to establish the source of infection. If badgers are implicated, badger removal is conducted under licence by the Department of Agriculture, Food and the Marine. From the late 1980's until 2004, the badger removal program comprised of the removal of badgers as a single event from setts found within 1 kilometre of the breakdown herd. Since 2004, the method of badger removal changed from this single removal event to a continued removal of badgers from setts (main setts 1.5 kilometres and other setts 2km from the breakdown herd). This study aims to compare the effectiveness of these two badger removal approaches by examining their comparative survival times following the initial badger removals. Severity of future breakdowns could also be used as a measure of determining effectiveness. The study could be expanded to include the effect on neighbouring farms and/or farms up to a defined distance.

Risk-based badger removal and the subsequent herd breakdown rate for Bovine Tuberculosis in Co. Monaghan, Ireland

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For the purposes of this study, a herd is classed as an "index herd" where, following an ER76 epidemiological investigation into the source of a bTB breakdown, approval is granted for local badger removal. This study aims to assess the effect of local badger removal on the risk of a next herd bTB breakdown among neighbouring herds surrounding an index herd. To achieve this, we will conduct a survival analysis with the outcome being time to next breakdown for herds surrounding setts from which badgers were removed under the Wildlife Unit program. Herd exposure is measured as the proportion of a herd(s) land area within 1km of any sett removal. We will test the hypothesis that the hazard of bTB for herds with a high percentage of land area within 1km of sett removal do not differ from herds with a low percentage of land area within 1km of sett removal.

Progress towards a badger vaccine

Badger tuberculosis vaccine

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a. Vaccine development (studies with captive badgers)

The first phase of research into BCG vaccination against tuberculosis in captive badgers, an integrated series of seven experiments and associated studies that commenced in 2001, has been completed. These studies have established as proof of principle that vaccination of badgers with BCG, when delivered by a variety of routes, can protect badgers against tuberculosis. The BCG vaccine has been chosen for use based on its availability, low production cost and much experience of its application in domestic and wild animals, and humans. An oral vaccine developed by Dr. Frank Aldwell, Otago University, New Zealand, has been shown to work in captive badgers and paves the way for development of oral delivery systems for use in wild badgers. In our most recent study, we have shown that the oral vaccine is effective against challenge with a low dose of *M. bovis*, at levels likely to be encountered under conditions of natural transmission. The data has given us a much better understanding of how the vaccine is likely to perform under natural conditions and will facilitate the development of strategies to deliver the vaccine to badger populations. As part of these studies, and working closely with colleagues at AHVLA (Weybridge UK), we helped develop and assess a range of *in vitro* diagnostic assays based on the immunological responses of badgers to challenge with *M. bovis*.

b. The vaccine field trial

The success of the captive badger studies has paved the way for the testing of oral BCG vaccine in a large-scale field trial. The BCG vaccine field trial has two principal objectives. These are to validate the results of captive badger studies and show that BCG vaccine is protective in naturally exposed wild badgers, and to estimate vaccine efficacy under field conditions. The aim of the field trial is to estimate the efficacy of BCG vaccine and to demonstrate protection in a wild badger population by comparing prevalence of tuberculosis in vaccinated and non-vaccinated badgers after three years. A secondary outcome of the field trial will be to measure the effect of BCG vaccine in badgers with pre-existing *M. bovis* infection. In addition to providing a measurement of protection and an estimate of vaccine efficacy, the field trial will provide a practical basis for understanding the logistics of oral vaccine delivery to wild badger populations.

The first capture sweep commenced in September 2009 and, to date, five capture sweeps of the vaccine field trial has been completed. The trial area of approx. 755 square kilometres is divided into three zones (Figure 1) each with a different level of vaccine coverage. Vaccine and placebo are blind coded and field staff are unaware of the vaccine status of the badgers they are treating. Badgers captured in the middle zone are randomly assigned vaccine or placebo on approximately a 50:50 basis, 100% of badgers captured in the north and south will receive either vaccine or placebo therefore there will be a gradient of vaccine cover from 0% to 50% to 100% vaccination from north to south or south to north.

- The entire trial area has been trapped for the fifth time over Autumn/Winter 2011
- During the fifth sweep of the trial area, badgers were captured and examined on 250 occasions (Table 1)
- The total treatments to date across the three zones since start of field trial is shown in Table 2



Figure 1 – Outline of vaccine trial area and divisions into three zones

Table 1. Age and sex of the badgers examined (n = 250) in sweep 5 of the vaccine trial

	Cub	Juvenile	Adult	Old	Total
Female	0	38	69	19	126
Male	0	34	82	8	124
Total	0	72	151	27	250

Table 2. Treatment to date in the three zones of the trial area*

	1*	2*	Total**
Zone A	0	408	408
Zone B	99	108	207
Zone C	369	1	370
Total	468	517	985

* Badgers receive either vaccine or placebo, blind-coded 1 & 2

** Total includes 208 badgers that were revaccinated in sweep 3, 4 and 5

At the end of the 3 year study period (Aug 2012), the trial site will be depopulated and all badgers will be examined for tuberculosis by detailed post mortem examination that will include an examination for visible lesions, histologic lesions, and mycobacteriology. The isolation of *M. bovis* from post mortem or clinical samples (wound exudates or tracheal swabs) will be used to define a case of tuberculosis. The results and experience gained from the field trial will facilitate the development of strategies for introduction of vaccination into the national program.

c. Development of badger immunodiagnostics

We have continued to develop and assess a range of immunodiagnostic tests that will be required for tuberculosis surveillance in badger populations to monitor the effect of vaccination. This work, carried out in collaboration with AHVLA Weybridge and with Enfer group, is focused on developing cell-mediated immune and antibody based diagnostic tests for badgers. To date, we have established that the sensitivity of all the immunodiagnostic assays improves as the disease severity increases and that this is more pronounced with the serological based assays. The serology tests are being applied to the field trial blood samples and will assist in the validation of the tests on naturally infected badgers.

Trial design to estimate the effect of vaccination on tuberculosis incidence in badgers

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Veterinary Microbiology 151, 104-111 (2011)

The principal wildlife reservoir of *Mycobacterium bovis* in Ireland is the European badger. Studies in the Republic of Ireland (RoI) have shown that badgers culled in association with cattle herd tuberculosis breakdowns (focal culling) have a higher prevalence of infection than the badger population at large. This observation is one rationale for the medium term national strategy of focal badger culling. A vaccination strategy for the control of bovine tuberculosis (bTB) in badgers is a preferred long-term option. The Bacillus Calmette-Guérin (BCG) vaccine has been shown to decrease disease severity in captive badgers under controlled conditions. As the vaccine has been tested in a controlled environment with precise information on infection pressure, it cannot be assumed *a priori* that the effects of vaccination are similar in the wild, where other environmental and/or ecological factors prevail. For this reason we have designed a vaccine field trial to assess the impact of vaccination on the incidence of TB infection in a wild badger population. The selected study area for the vaccine trial (approximately 755 square kilometers) is divided into three zones each of which has similar characteristics in terms of size, number of main badger setts, cattle herds, cattle and land classification type. Three vaccination levels (100%, 50% and 0%) will be allocated to the three zones in a way that a gradient of vaccination coverage North to South is achieved. The middle zone (zone B) will be vaccinated at a 50% coverage but zone A and C will be randomly allocated with 100% or 0% vaccination coverage. Vaccination within zone B will be done randomly at individual badger level. The objective of this paper is to describe the design of a field tuberculosis vaccination trial for badgers, the epidemiological methods that were used to design the trial and the subsequent data analysis. The analysis will enable us to quantify the magnitude of the observed vaccination effect on *M. bovis* transmission in badgers under field conditions and to improve our knowledge of the biological effects of vaccination on susceptibility and infectiousness.

Reprinted from Veterinary Microbiology, 151, Aznar, I., McGrath, G., Murphy, D., Corner, L.A.L., Gormley, E., Frankena, K., More, S.J., Martin, W., O'Keeffe, J., De Jong, M.C.M., Trial design to estimate the effect of vaccination on tuberculosis incidence in badgers, 104-111, Copyright 2011, with permission from Elsevier.

Using simulation to estimate the power of a badger vaccine trial against *Mycobacterium bovis* in badgers

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The aim of this study was to estimate the power of a badger vaccine field trial designed in the Republic of Ireland to help in the control of bovine tuberculosis using simulation techniques. The effects of sample size (recapture percentage), sensitivity and specificity of the diagnostic test, transmission rate between unvaccinated badgers, Vaccine Efficacy for Susceptibility (VES) and Vaccine Efficacy for Infectiousness (VEI), on study power were determined. Sample size had a small effect on power. Study power increased with increasing transmission rate between non-vaccinated badgers. Changes in VES had a higher impact on power than changes in VEI. However, the largest effect on study power was seen by changes in the specificity of the diagnostic test. The effect of changes in sensitivity on study power was much lower. Therefore, it is critical that the diagnostic test used in the badger vaccine trial is optimized to maximise test specificity.

Infection dynamics and effective control strategies of tuberculosis in badgers and cattle of Ireland

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The main objective is to assess the impact of interventions on bovine tuberculosis (bTB) prevalence in cattle and badgers; for this a mathematical model of bTB transmission that describes the disease in cattle and badgers in the Republic of Ireland will be developed. The hypothesis is that control, with the ultimate goal of eradication, cannot be achieved until badger-to-cattle transmission is effectively addressed. Data from ongoing field trials (particularly data related to efficacy of the badger vaccine) and data from previous research work will be utilized to inform parameter estimation. Experimental work (the exact nature of which will depend on identified gaps in the knowledge required for parameter estimation) will also be carried out.

Optimisation of a multiple antigen ELISA test to be used in a badger vaccine trial

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The main objective of this study was to optimize a diagnostic test for *M. bovis* infection in live badgers trapped during the badger vaccine trial in Ireland using a multiplex chemiluminiscent assay (Enfer Scientific). A total of 215 blood samples (including 200 blood samples used by Whelan *et al.*, 2009) were tested against a panel of 8 *M. bovis* antigens. The optimization was done while keeping specificity at 99.99% to allow for a reasonable power (60-80%) to be obtained in the vaccine trial. We also aimed to explore the effects of vaccination on test characteristics and review the implications for analysis of the data obtained from the Kilkenny badger vaccine trial.



Bacillus Calmette-Guérin vaccination reduces the severity and progression of tuberculosis in badgers

Chambers, M.A.¹, Rogers, F.^{1,2}, Delahay, R.J.², Lesellier, S.¹, Ashford, R.¹, Dalley, D.¹, Gowtage, S.¹, Davé, D.¹, Palmer, S.¹, Brewer, J.¹, Crawshaw, T.¹, Clifton-Hadley, R.¹, Carter, S.², Cheeseman, C.², Hanks, C.², Murray, A.², Palphramand, K.², Pietravalle, S.², Smith, G.C.², Tomlinson, A.², Walker, N.J.², Wilson, G.J.², Corner, L.A.L.³, Rushton, S.P.⁴, Shirley, M.D.F.⁴, Gettinby, G.⁵, McDonald, R.A.², Hewinson, R.G.¹

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Proceedings of the Royal Society B 278, 1913-1920 (2011)

Control of bovine tuberculosis (TB) in cattle has proven particularly challenging where reservoirs of infection exist in wildlife populations. In Britain and Ireland, control is hampered by a reservoir of infection in Eurasian badgers (*Meles meles*). Badger culling has positive and negative effects on bovine TB in cattle and is difficult, costly and controversial. Here we show that *Bacillus Calmette-Guérin* (BCG) vaccination of captive badgers reduced the progression, severity and excretion of *Mycobacterium bovis* infection after experimental challenge. In a clinical field study, BCG vaccination of free-living badgers reduced the incidence of positive serological test results by 73.8 per cent. In common with other species, BCG did not appear to prevent infection of badgers subjected to experimental challenge, but did significantly reduce the overall disease burden. BCG vaccination of badgers could comprise an important component of a comprehensive programme of measures to control bovine TB in cattle.

Reprinted from Proceedings of the Royal Society B, 278, Chambers, M.A., Rogers, F., Delahay, R.J., Lesellier, S., Ashford, R., Dalley, D., Gowtage, S., Davé, D., Palmer, S., Brewer, J., Crawshaw, T., Clifton-Hadley, R., Carter, S., Cheeseman, C., Hanks, C., Murray, A., Palphramand, K., Pietravalle, S., Smith, G.C., Tomlinson, A., Walker, N.J., Wilson, G.J., Corner, L.A.L., Rushton, S.P., Shirley, M.D.F., Gettinby, G., McDonald, R.A., Hewinson, R.G., Bacillus Calmette-Guérin vaccination reduces the severity and progression of tuberculosis in badgers, 1913-1920, Copyright 2011, with permission from The Royal Society.

Oral vaccination of badgers (*Meles meles*) with BCG and protective immunity against endobronchial challenge with *Mycobacterium bovis*

Corner, L.A.L.¹, Costello, E.², O'Meara, D.², Lesellier, S.^{1,5}, Aldwell, F.E.³, Singh, M.⁴, Hewinson, R.G.⁵, Chambers, M.A.⁵, Gormley, E.¹

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Vaccine 28, 6265-6272 (2010)

Eurasian badgers (*Meles meles*) are a reservoir host of *Mycobacterium bovis* and are implicated in the transmission of tuberculosis to cattle in Ireland and Great Britain. The development of a vaccine for use in badgers is considered a key element of any long-term sustainable campaign to eradicate the disease from livestock in both countries. The aim of this study was to investigate the protective response of badgers vaccinated orally with Bacille Calmette-Guérin (BCG) encapsulated in a lipid formulation, followed by experimental challenge with *M. bovis*. A group of badgers was vaccinated by inoculating the BCG-lipid mixture containing approximately 10⁸ colony forming units (cfu) of BCG into the oesophagus. The control group was sham inoculated with the lipid formulation only. Thirteen weeks after vaccination

all the badgers were challenged with approximately 10^4 cfu of *M. bovis* delivered by endobronchial inoculation. Blood samples were taken throughout the study and the cell mediated immune (CMI) responses in peripheral blood were monitored by the IFN-gamma ELISA and ELISPOT assay. At 17 weeks after infection all the badgers were examined post mortem to assess the pathological and bacteriological responses to challenge. All badgers in both groups were found to be infected. However, a significant protective effect of BCG vaccination was measured as a decrease in the number and severity of gross lesions, lower bacterial load in the lungs, and fewer sites of infection. The analysis of immune responses showed that vaccination with BCG did not generate any detectable CMI immunological responses, however the levels of the responses increased in both groups following *M. bovis* infection. The results of the study showed that vaccination with oral BCG in the lipid formulation generated a protective effect in the badgers.

Reprinted from Vaccine, 28, Corner, L.A.L., Costello, E., O'Meara, D., Lesellier, S., Aldwell, F.E., Singh, M., Hewinson, R.G., Chambers, M.A., Gormley, E., Oral vaccination of badgers (Meles meles) with BCG and protective immunity against endobronchial challenge with Mycobacterium bovis, 6265-6272, Copyright 2010, with permission from Elsevier.

Control of tuberculosis in badgers by vaccination: Where next?

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The Veterinary Journal 189, 239-241 (2011)

With the development of an oral vaccine for use in badgers continuing, this paper examines a range of strategic options available for vaccination that might be employed to control and eradicate TB in badgers, and the beneficial impact this will have in cattle herds.

Adapted from The Veterinary Journal, 189, Gormley, E., Corner, L.A.L., Control of tuberculosis in badgers by vaccination: Where next?, 239-241, Copyright 2011, with permission from Elsevier.

Evaluation of attractant flavours for use in oral vaccine baits for badgers (*Meles meles*)

Kelly, D.J.¹, Corner, L.A.L.², Gormley, E.², Murphy, D.², Costello, E.³, Aldwell, F.E.⁴, Marples, N.M.¹

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European Journal of Wildlife Research 57, 767-774 (2011)

European badgers (*Meles meles*) are a wildlife reservoir for *Mycobacterium bovis* infection (tuberculosis) in Ireland and the UK and are implicated in the transmission of infection to livestock. Vaccination of badgers with the human BCG vaccine (Bacille Calmette Guérin) is considered as an important strategy to reduce the burden of disease in this species, and a pragmatic approach is likely to involve oral vaccination. In this study, we evaluated nine different flavours for use as attractants in a prototype oral vaccine bait for European badgers (*M. meles*): aniseed, apple, cocoa powder, carob powder, curry, fish, garlic, peanut and strawberry. The bait matrix was composed of a natural lipid formulation, developed as a vehicle for oral vaccination against tuberculosis in wildlife. A 'food for work' paradigm was employed during the trials to ensure the animals were actively seeking the baits. The trials showed carob and cocoa powders were equally attractive and more attractive than any of the other candidates. Carob and cocoa show potential as bait attractants for badgers and might form part of a novel vaccine delivery system.

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The development of a bait delivery system for oral vaccination of badgers against tuberculosis

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This work has identified the potential of carob as an attractant for use with oral vaccine baits and has established the importance of pre-feeding when introducing "flapjack" baits to "naïve" Irish badger populations. We have found that pre-feeding was most effective when the trial baits were most similar to the pre-fed baits, and that packaged baits can be ignored if they are not coated in an attractant. The results also suggest that burying baits may stimulate natural feeding behaviours. This could imply that buried baits may be more attractive to badgers than baits placed in sett entrances, despite the latter being more easily available. The development of a bait for oral vaccination is continuing.

Protection of Eurasian badgers (*Meles meles*) from tuberculosis after intra-muscular vaccination with different doses of BCG

Lesellier, S.¹, Palmer, S.¹, Gowtage-Sequiera, S.¹, Ashford, R.¹, Dalley, D.¹, Davé, D.¹, Weyer, U.¹, Salguero, F.J.¹, Nuñez, A.¹, Crawshaw, T.², Corner, L.A.L.³, Hewinson, R.G.¹, Chambers, M.A.¹

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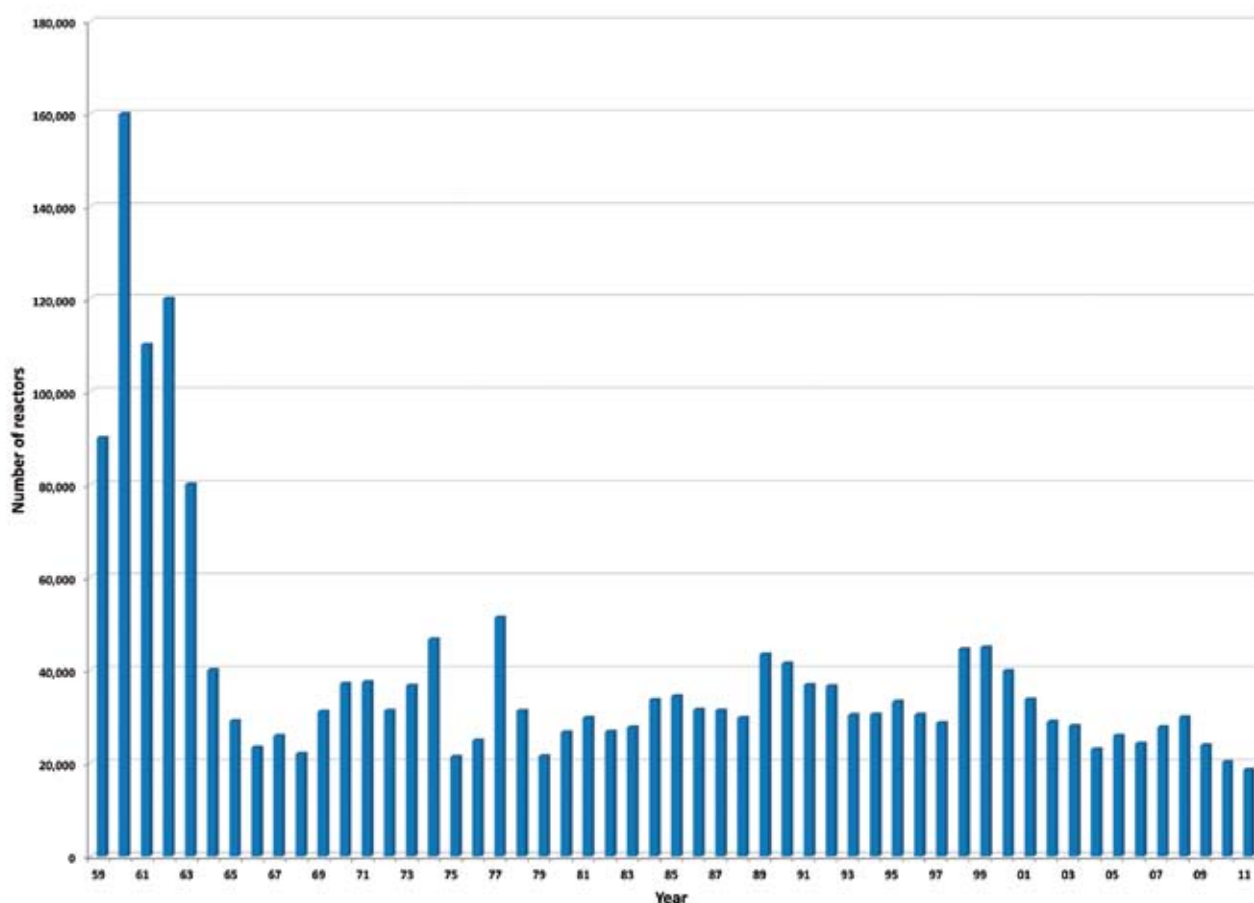
³ UCD School of Veterinary Medicine

Vaccine 29, 3782–3790 (2011)

Mycobacterium bovis infection is widespread in Eurasian badger (*Meles meles*) populations in Great Britain and the Republic of Ireland where they act as a wildlife reservoir of infection for cattle. Removal of infected badgers can significantly reduce the incidence of bovine tuberculosis (TB) in local cattle herds. However, control measures based on culling of native wildlife are contentious and may even be detrimental to disease control. Vaccinating badgers with Bacillus Calmette-Guérin (BCG) has been shown to be efficacious against experimentally induced TB of badgers when administered subcutaneously and orally. Vaccination may be an alternative or complementary strategy to other disease control measures. As the subcutaneous route is impractical for vaccinating wild badgers and an oral vaccine bait formulation is currently unavailable, we evaluated the intramuscular (IM) route of BCG administration. It has been demonstrated that the IM route is safe in badgers. IM administration has the practical advantage of being relatively easy to perform on trapped wild badgers without recourse to chemical immobilisation. We report the evaluation of the efficacy of IM administration of BCG Danish strain 1331 at two different doses: the dose prescribed for adult humans ($2\text{--}8 \times 10^5$ colony forming units) and a 10-fold higher dose. Vaccination generated a dose-dependent cell-mediated immune response characterised by the production of interferon- γ (IFN γ) and protection against endobronchial challenge with virulent *M. bovis*. Protection, expressed in terms of a significant reduction in the severity of disease, the number of tissues containing acid-fast bacilli, and reduced bacterial excretion was statistically significant with the higher dose only.

Reprinted from Vaccine, 29, Lesellier, S., Palmer, S., Gowtage-Sequiera, S., Ashford, R., Dalley, D., Davé, D., Weyer, U., Salguero, F.J., Nuñez, A., Crawshaw, T., Corner, L.A.L., Hewinson, R.G., Chambers, M.A., Protection of Eurasian badgers (*Meles meles*) from tuberculosis after intra-muscular vaccination with different doses of BCG, 3782–3790, Copyright 2011, with permission from Elsevier.

The national programme



The number of TB reactors detected in Ireland each year between 1959 and 2011.

Progress in tuberculosis eradication in Ireland

Sheridan, M.¹

¹ DAFM

Veterinary Microbiology 151, 160-169 (2011)

Ireland ran a conventional test and slaughter Bovine Tuberculosis eradication programme from 1954 until 1988. This programme fulfilled our trading requirements but failed to eradicate TB. At this point a major initiative, ERAD, was launched targeted with reducing the disease levels by half within a four-year period and devising the strategy and supports necessary to achieve final eradication. The lessons learned at that time have informed Ireland's eradication programme ever since. Eradication was not possible without developing solutions to address the wildlife disease reservoir and other identified constraints. Since 1992 the programme objectives have been restated. It is now effectively an interim control programme where significant resources have been invested in research and development aimed at overcoming the identified constraints to eradication. Policy is informed by science and debate among stakeholders is generally knowledgeable and balanced. This paper outlines developments in recent years and sets out our expectations for progress in the period ahead.

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Perspectives on the history of bovine TB and the role of tuberculin in bovine TB eradication

Good, M.¹, Duignan, A.¹

¹ DAFM

Veterinary Medicine International, article ID 410470 (2011)

Tuberculosis remains a significant disease of animals and humans worldwide. Bovine tuberculosis is caused by *Mycobacteria* with an extremely wide host range and serious, although currently probably underdiagnosed, zoonotic potential. Where bovine tuberculosis controls are effective, human zoonotic TB, due to *Mycobacterium bovis* or *M. caprae*, is uncommon and clinical cases are infrequent in cattle. Therefore, the control and ultimate eradication of bovine tuberculosis is desirable. Tuberculin tests are the primary screening tool used in bovine eradication. The choice of tuberculin test is dependent on the environment in which it is to be used. Tuberculin potency is critical to test performance, and the accurate determination of potency is therefore particularly important. The design of a control or eradication programme should take into consideration the fundamental scientific knowledge, the epidemiological profile of disease, the experience of other eradication programmes, and the presence, in the same ecosystem, of maintenance hosts, in which infection is self-sustaining and which are capable of transmitting infection. A control or eradication programme will necessarily require modification as it progresses and must be under constant review to identify the optimal desirable goals, the efficacy of policy, and constraints to progress.

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Management of the Irish TB eradication programme: the development and application of new measures of performance

Higgins, I.M.¹, Williams, D.H.², More, S.J.^{1,3}

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Performance management in national TB programmes has not been straight-forward. There is a broad range of reasons for this, including infection epidemiology, test interpretation and data collection and management. As one example of the complexity involved, although TB-related data in Ireland are collected at the level of the event (test, breakdown), some relate to the animal (which is nested within herd) and some to the herd. In Ireland, national progress is currently measured using the number of reactors per 1,000 tests (APT), which is computationally simple, however, there are a number of difficulties with this approach. This paper describes work to define performance measures to assist with ongoing review of the Irish programme, to create automated methods to enable ongoing measurement, and to review progress. A range of performance measures, linked to defined periods of herd trading restriction following indications of *M. bovis* infection, have been developed to separately assess the effectiveness of surveillance (to detect new cases: herd disease incidence, percentage of herds remaining disease free) and control (to clear infection following case detection: restriction duration, number of reactors per restriction, % single reactor breakdowns, % 3rd/4th reactor retests, inter-episode interval, repeat restrictions). Programming was conducted using SAS® (SAS Institute Inc., Cary, NC, USA) with extensive use of SAS® macro programming, using TB herd summary results from the national Animal Health Computer System (AHCS) as data inputs. The final set of programmes can be run without intervention. The surveillance and control measures have been calculated for all active Irish herds, and summarized by District Veterinary Office (unit of programme management), production type, herd size and past disease history. In contrast to current methods, these herd-level measures effectively

partition activities in Ireland relating to detection of new cases (surveillance) and the resolution of cases following detection (control). This information, provided on an ongoing and timely basis will be of benefit to national and regional programme decision-makers.

TB in Ireland - visualising the current picture

McGrath, G.¹

¹ UCD CVERA

The current methodologies used to visualise the prevalence of tuberculosis in Ireland include thematic mapping (positive animal tests per thousand tests per district electoral division) and density maps (kernel densities of positive animals/population). This project will investigate possible new ways of displaying the changes in tuberculosis prevalence over time to provide a better visualisation of the recent decrease in reactor numbers. A methodology is required that is more sensitive to representing proportionally small changes. The proposed method will involve assigning herd level data to a uniform hexagonal grid. A measure of mean prevalence will be calculated for a defined period of time. Each year will then be displayed as a deviation above or below this mean level thus showing the relative temporal trend in prevalence for each unit through time.

An evaluation of the Irish Single Reactor Breakdown Protocol for 2005 to 2008 inclusive and its potential application as a monitor of tuberculin test performance

Good, M.¹, Duignan, A.¹

¹ DAFM

Veterinary Microbiology 151, 85-90 (2011)

Under the Irish Bovine Tuberculosis (bTB) Eradication Programme all herds are subjected to at least one test *per annum*. The Single Intradermal Comparative Tuberculin Test (SICTT) is used in Ireland for the detection of cattle infected with *Mycobacterium bovis*. There have been concerns regarding the specificity of the SICTT, notably by farmers, and particularly in herds where the detection of a single positive animal in the absence of an obvious source of (bTB) infection could be perceived as a “false” positive. To address this issue the so-called ‘Singleton Protocol’ was established as part of the Irish bTB eradication programme. This protocol allows for the early restoration of free trading status to herds where a single positive animal was detected and where the herd was not confirmed as infected with *M. bovis* by epidemiological investigation, by post mortem, by laboratory examination, or by further test. This paper presents data from the 2005 to 2008, inclusive, bTB programmes on the number of herds that were assessed, which qualified for inclusion under the ‘Singleton Protocol’ and the outcome for qualifying herds up to and including having status restored early as a consequence of inclusion in that programme. The outcome of this protocol reaffirms the reliability of the SICTT at current levels of infection. Furthermore it is advocated that the ‘Singleton Protocol’ be continued as a monitor of herds in which a single positive animal is disclosed, and as overall infection levels of bTB fall the outcome may be used as one means to assess progress towards bTB eradication in Ireland.

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Singleton area risk: Can areas of temporo-spatial clustering in singleton reactors be defined?

McGrath, G.¹

¹ UCD CVERA

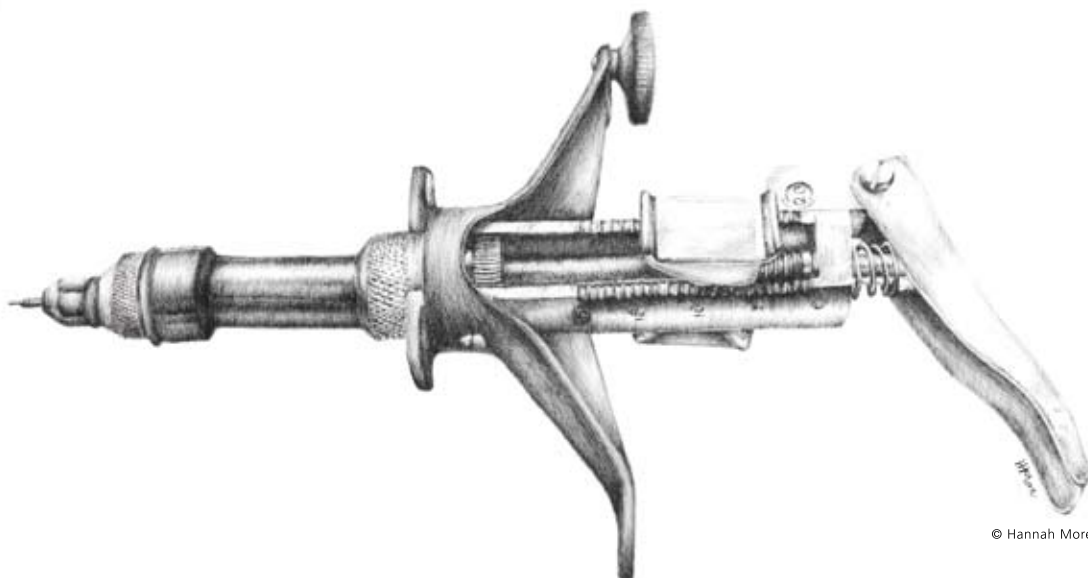
There is evidence that non-specific infection or singleton reactors occur as a result of environmental conditions found on farms close to bog, reclaimed wetland and lakes. If this is the case, they should cluster in space and time. We would expect there to be a spatial consistency which should be discernible. The objective of this study is to attempt to identify if this spatial/temporal clustering of singleton reactors exists. If there is a discernible effect, areas of persistent high singleton risk could be defined and assigned back to all herds falling within these areas providing veterinary inspectors with an additional layer of information to aid in deciding how to interpret and manage herds with singleton reactors.

Evaluation of single reactor bovine tuberculosis breakdowns, based on analysis of reactors slaughtered at an Irish export meat plant

Murray, D.¹, Clegg, T.A.², More, S.J.^{2, 3}

¹ DAFM, ² UCD CVERA, ³ UCD School of Veterinary Medicine

The 'Singleton Protocol' was adopted by the Irish Department of Agriculture, Food and the Marine (DAFM) in 1996 to address the incomplete specificity of the Single Intradermal Comparative Tuberculin Test (SICTT) used in Ireland for the detection of animals infected with bovine tuberculosis (bTB). The protocol allows the early restoration of disease-free status to herds with a single reactor breakdown, where the herd was not confirmed as infected with *Mycobacterium bovis* by epidemiological investigation, by post mortem examination or by further test. The current study examines the ability of the Singleton Protocol to identify false positive reactors, the subsequent herd reactor rate following single reactor removal and analyses the factors leading to a positive post mortem lesion outcome and a positive reactor retest result. Post mortem lesion results were obtained for 371 reactor animals from single reactor breakdowns that were killed at an export meat plant over a nineteen-month period. Epidemiological and test data for these animals and their herds were obtained from DAFM databases and analysed by univariate and multivariate statistical analysis. Singleton candidates had an 18.7% lower lesion rate than single animal breakdowns not meeting the singleton criteria. No significant difference was found between singletons and non-singletons in the subsequent reactor retest results. Skin thickness at the SICTT is the most significant determinant of a positive lesion result. The area bTB history was shown to be a significant variable in producing a positive reactor retest result.



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Bovine tuberculosis trends in the United Kingdom and Republic of Ireland, 1995 to 2010

Abernethy, D.A.¹, Upton, P.², Higgins, I.M.³, McGrath, G.³, Goodchild, T.², Rolfe, S.⁴, Broughan, J.², Downes, S.², Clifton-Hadley, R.², Menzies, F.¹, de la Rua-Domenech, R.⁵, Blissit, M.⁶, Duignan, A.⁷, More, S.J.^{3,8}

¹ Department of Agriculture and Rural Development, Belfast, Northern Ireland, ² Animal Health and Veterinary Laboratories Agency, Weybridge, United Kingdom ³ UCD CVERA, ⁴ Office of the Chief Veterinary Officer, Welsh Assembly, Cardiff, Wales, ⁵ Department for Environment, Food and Rural Affairs, London, England, ⁶ Scottish Government, Edinburgh, Scotland, ⁷ DAFM, ⁸ UCD School of Veterinary Medicine

European legislation, principally 64/432 EEC and 78/52 EEC, forms the basis of national programmes and governs the surveillance and control measures applied by Member States. The United Kingdom includes the countries of Northern Ireland, England, Wales and Scotland. The latter three are collectively referred to as Great Britain while Northern Ireland is located with the Republic of Ireland on the island of Ireland, separated from Great Britain by the Irish Sea. Historically, bTB policy within the United Kingdom was determined separately for Great Britain and Northern Ireland, leading to differences in strategy and management. Devolution of responsibility for agriculture to the Welsh and Scottish administrations in 1998 further regionalised policy development. Thus, although programme measures were largely standardised through European legislation, some differences occurred, largely in response to specific risks or bTB prevalence. Comparing bTB trends provides an opportunity to learn from differing experiences and gain possible insight into factors that may promote or impede progress towards eradication. Such comparisons, however, are hampered by differences in measurement of disease parameters and summary statistics. This project will seek to identify and wherever possible, reduce these differences to allow direct comparison of trends between the jurisdictions.



Photograph by E. Gormley.

Bovine tuberculosis in Europe from the perspective of an officially tuberculosis - free country: Trade, surveillance and diagnostics

Schiller, I.¹, Waters, W.R.², Vordermeier, H.M.³, Jemmi, T.¹, Welsh, M.⁴, Keck, N.⁵, Whelan, A.³, Gormley, E.⁶, Boschioli, M.L.⁷, Moyen, J.L.⁸, Vela, C.⁹, Cagiola, M.¹⁰, Buddle, B.M.¹¹, Palmer, M.², Thacker, T.², Oesch, B.¹²

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³ Veterinary Laboratory Agency, Addlestone, United Kingdom, ⁴ AFBI-Veterinary Sciences Division, Stormont, Northern Ireland, ⁵ Laboratoire Départemental Vétérinaire de l'Hérault, Montpellier, France, ⁶ UCD School of Veterinary Medicine, ⁷ Unité de Zoonoses Bactériennes, AFSSA-LERPAZ, Maisons-Alfort, France,

⁸ Laboratoire Conseil Général de la Dordogne, France, ⁹ Ingenasa, Madrid, Spain, ¹⁰ Istituto Zooprofilattico dell'Umbria e delle Marche, Perugia, Italy,

¹¹ AgResearch, Palmerston North, New Zealand, ¹² Prionics AG, Schlieren, Switzerland

Veterinary Microbiology 151, 152-159 (2011)

Switzerland has been officially free of bovine tuberculosis (OTF) since 1960. Since 1980 the control of bovine tuberculosis (bTB) has been reduced to passive abattoir surveillance. Isolated cases of bTB, partly due to reactivation of human *Mycobacterium bovis* infections with subsequent transmission to cattle, have been noticed in the last years. In Europe, the overall prevalence of bTB is slightly increasing. Both OTF and non-OTF countries report increases in the proportion of bTB positive cattle herds. Current bTB eradication and control programs in Europe are facing a range of challenges. Whole herd depopulation is becoming a less attractive option for economic reasons and due to animal welfare concerns. Live animal trade is increasing both at national and international levels. Regarding these tendencies and taking into account the chronicity of bTB infection, pre-movement testing is becoming increasingly important as a central tool for eradication and for protection against re-introduction of bTB. Pre-movement testing, however specifically focuses on the infection status of individuals, requiring a high level of diagnostic accuracy to correctly diagnose infected animals. Current screening tests for bTB, however, have been designed to meet demands as herd tests. This illustrates that the modification of existing and/or the development of new diagnostics for bTB might be needed. The tuberculin skin test (TST), the primary screening test for bTB may in certain situations have low sensitivity. The interferon gamma (IFN- γ) assay is accepted to be more sensitive compared to TST. Reduced specificity, however, especially in areas of low bTB prevalence raises concerns. New antigen combinations including Rv3615c, OmpATb and others have been shown to complement ESAT-6 and CFP-10 in the whole blood IFN- γ assay and resulted in improved sensitivity (compared to ESAT-6 and CFP-10) and specificity (compared to tuberculins). Lesion detection after slaughter represents a cost-effective procedure for passive surveillance of bTB, especially in areas of low prevalence or in regions free of bTB; however, its sensitivity is very low. This illustrates that trade is linked with a certain risk to re-introduce bTB in OTF regions or countries and that there may be delays in detecting a re-introduction of bTB. In conclusion, regarding the fact that some parameters linked with bTB programs are changing, the development of improved diagnostic tests with a high reliability for use as individual animal tests will be important for future eradication of bTB, in line with international commitment to high standard animal health programs.

Reprinted from Veterinary Microbiology, 151, Schiller, I., Waters, W.R., Vordermeier, H.M., Jemmi, T., Welsh, M., Keck, N., Whelan, A., Gormley, E., Boschioli, M.L., Moyen, J.L., Vela, C., Cagiola, M., Buddle, B.M., Palmer, M., Thacker, T., Oesch, B., Bovine tuberculosis in Europe from the perspective of an officially tuberculosis free country: trade, surveillance and diagnostics, 152-159, Copyright 2011, with permission from Elsevier.

Quality control

The relative effectiveness of, and reporting accuracy among, testers during field surveillance for bovine tuberculosis in Ireland

Clegg, T.A.¹, Good, M.², Duignan, A.², More, S.J.^{1, 3}

¹ UCD CVERA, ² DAFM, ³ UCD School of Veterinary Medicine

Field surveillance using the Single Intradermal Comparative Tuberculin Test (SICTT) is potentially problematic, noting its reliance on a range of factors, including the skills and experience of the tester. The objective of this study is to quantify the relative effectiveness of, and reporting accuracy among, testers during field surveillance for tuberculosis in Ireland. All testers who carried out at least one annual herd test in 2006 are included in the study. Relative testing effectiveness will be assessed by comparing the number of observed and expected herd restrictions per tester. The latter will be predicted, using a logistic regression model and testers will be ranked, based on the ratio of observed and expected restrictions. Reporting accuracy will be based on the bovine measurements in SICTT negative animals. The study will also be extended to look at 2007 to 2010 data and to compare changes in the ranking of testers over time.

A review of Irish tuberculin assays

Duignan, A.¹, Costello E.², Good M.¹, Kenny K.²

¹ DAFM, ² DAFM Veterinary Laboratory Service

Bovine tuberculin PPD has been described as a poorly defined, complex mixture containing more than 100 individual components in various stages of denaturation and is known to vary widely both in protein content and antigenic profile. The most reliable measure of the potency of a tuberculin is by assay in the species in which it will be routinely used. DAFM routinely conducts 2-3 potency assays on bovine tuberculin each year in cattle naturally infected with *M. bovis*. The trials are conducted by the DAFM Veterinary Laboratory Service on tuberculins chosen at random from the supply to be used in the field. The potency of a tuberculin is estimated by comparing the size of the reactions, elicited by an intradermal inoculation, to the size of the reactions of a 'standard' tuberculin of known potency. This project will give an outline of the evolution of PPD tuberculins and an account of assays carried out on Irish reactor cattle.

Quality control in the national bovine tuberculosis eradication programme in Ireland

Duignan, A.¹, Good, M.¹, More, S.J.^{2, 3}

¹ DAFM, ² UCD CVERA, ³ UCD School of Veterinary Medicine

The Irish Bovine Tuberculosis (bTB) eradication programme operates under national legislation and fulfils the requirements of the EU Trade Directive 64/432. The programme includes annual single intradermal comparative tuberculin test (SICTT) screening of all herds, prompt removal of test reactors and further consequential retesting of herds. Continuous evaluation of all relevant activities is essential to deliver an effective national programme and to reassure all stakeholders that the highest possible standards are attained. Quality control (QC) is a recognised process in the delivery of quality products or services. This project will present a review of QC in the bTB eradication programme in Ireland, with particular emphasis on field surveillance. A broad range of programme elements subjected to QC, will be described, including personnel, training, equipment, tuberculins and laboratory. Particular attention will be paid to field surveillance (specifically, Private Veterinary Practitioner (PVP) performance).

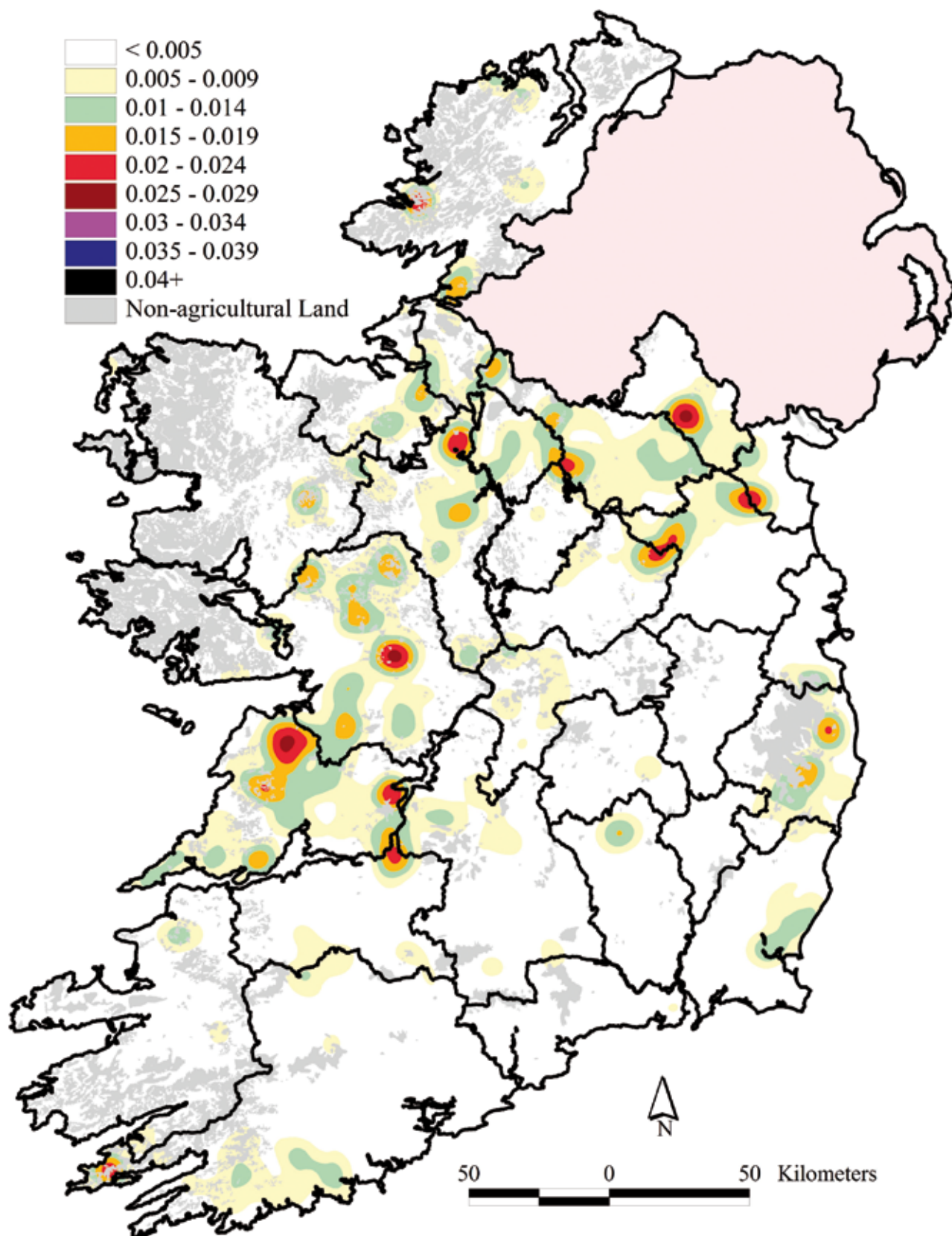
Description of opinions among stakeholders of factors affecting the quality of SICTT testing in Ireland

Meskell, P.¹, Mulreany, M.², More, S.J.^{3, 4}

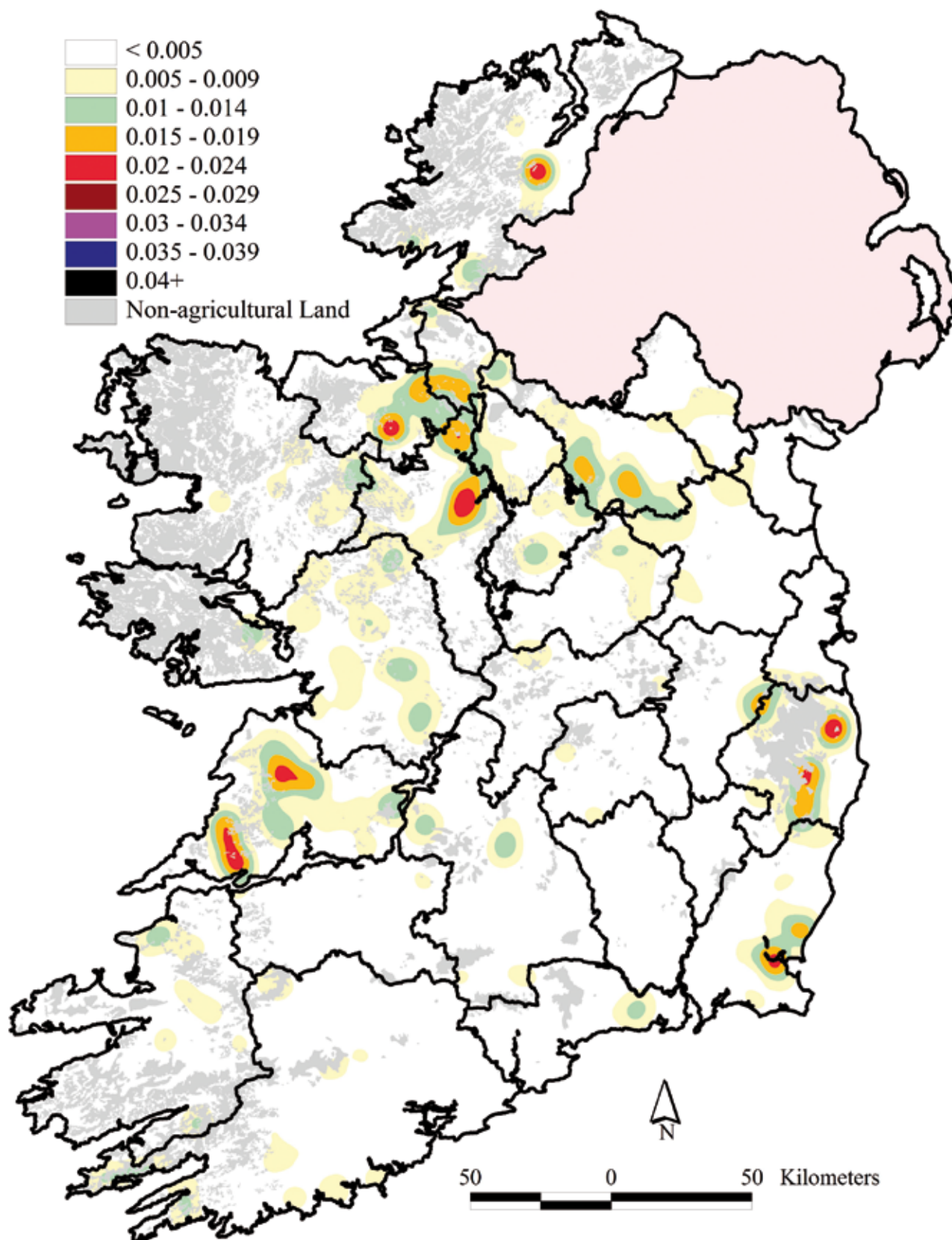
¹ DAFM, ² Institute of Public Administration, ³ UCD CVERA, ⁴ UCD School of Veterinary Medicine

The single intradermal comparative tuberculin test (SICTT) is the primary method of field surveillance for bovine tuberculosis (bTB) in Ireland, and a number of methods are used to safeguard testing quality. As yet, little has been documented about the opinions of stakeholders on this issue. The purpose of this study is to describe opinions among stakeholders of factors affecting the quality of SICTT testing in Ireland. The study is being conducted using several qualitative methods, including focus groups and semi-structured interviews.

Density of TB incidence

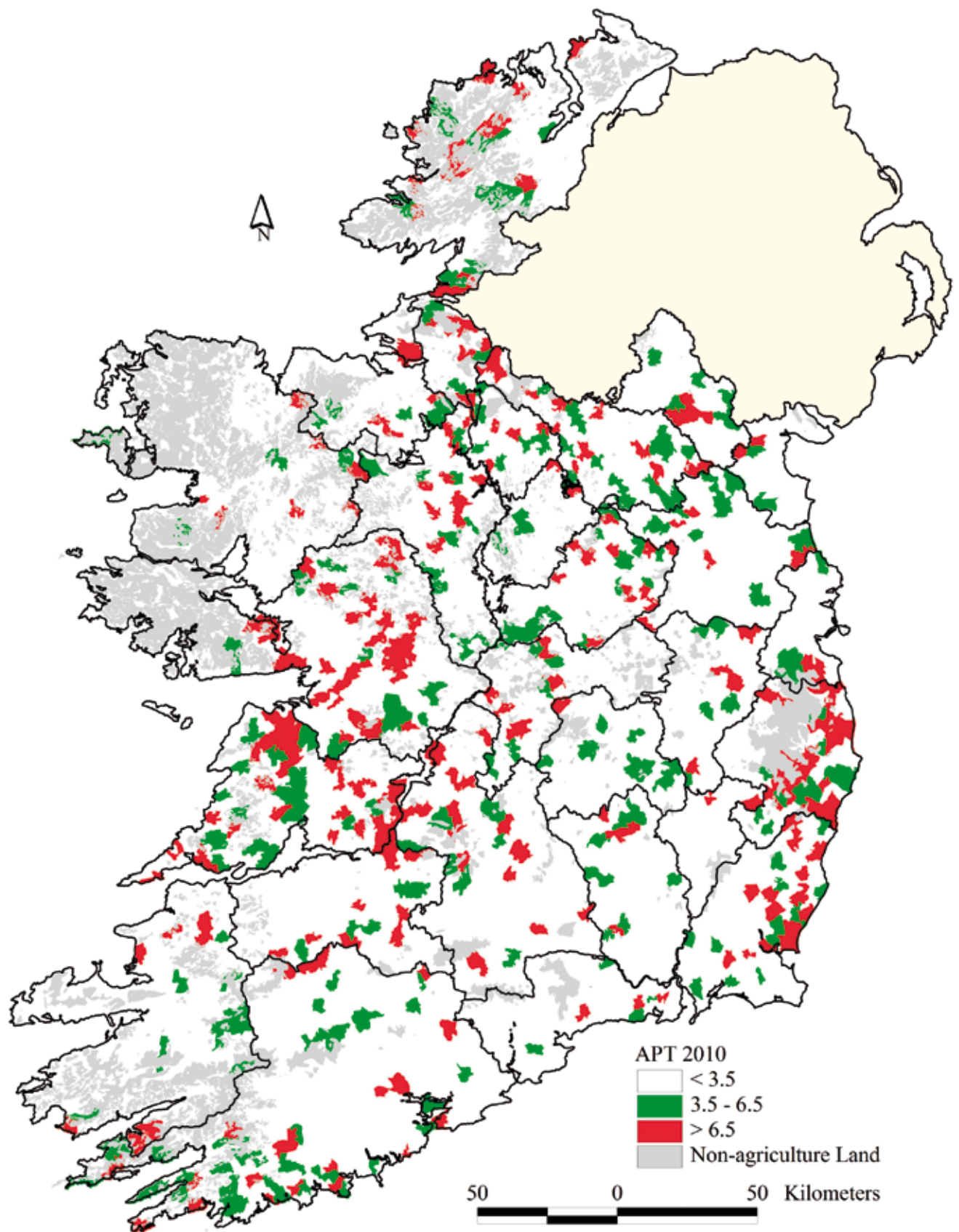


Density of TB incidence per square km during 2010 (kernel density with search radius at 10km).

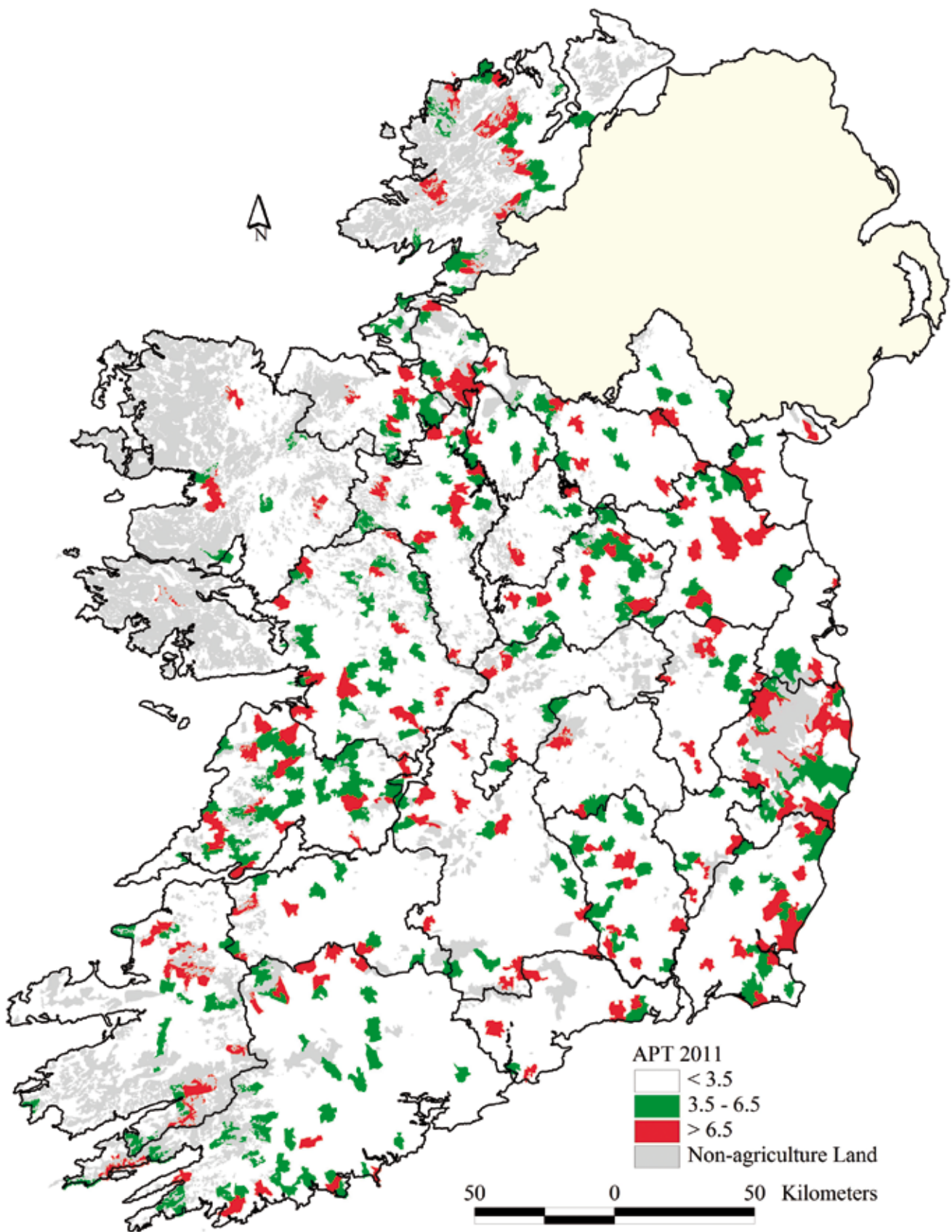


Density of TB incidence per square km during 2011 (kernel density with search radius at 10km).

APT per DED



APT (reactors per 1000 tests) per district electoral division, 2010.



APT (reactors per 1000 tests) per district electoral division, 2011.