



BOVINE TUBERCULOSIS

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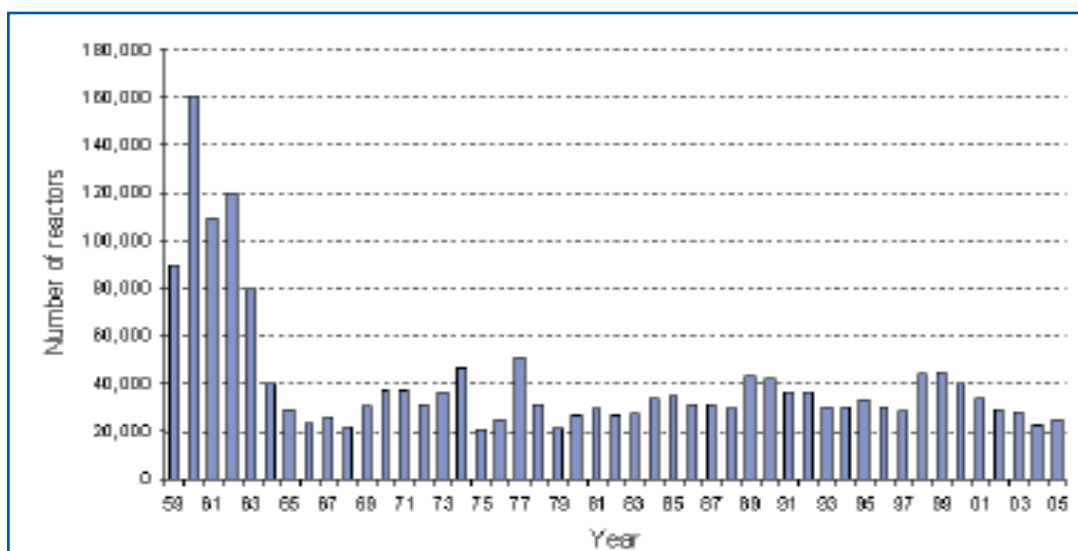
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Overview

The national bovine tuberculosis eradication programme in Ireland has been operating since 1954. During the initial stages of the programme, progress was rapid leading to a considerable reduction in disease prevalence by the mid-1960s. However, progress subsequently stalled, with between 20,000 and 50,000 reactors removed annually during the last 40 years.

Since 1988, the Department of Agriculture and Food has supported tuberculosis research within the UCD School of Agriculture, Food Science and Veterinary Medicine. The Tuberculosis Investigation Unit was established at this time with the aim 'to investigate the factors which militate against the eradication of tuberculosis ..., and to identify means to improve the present rate of eradication.' This support has now broadened to cover three complementary research programmes, including the Centre for Veterinary Epidemiology and Risk Analysis, the TB Diagnostics & Immunology Research Centre and the Badger Vaccination Programme.



Tuberculin reactors from 1959 to 2005

The Centre for Veterinary Epidemiology and Risk Analysis

The Centre for Veterinary Epidemiology and Risk Analysis (CVERA; originally the Tuberculosis Investigation Unit) is the national resource centre for veterinary epidemiology. Although its remit now covers a broad range of animal health issues, bovine tuberculosis remains a central part of its work.

During 2004-2005, the TB-related work of CVERA considered a range of key issues:

- *Clarifying the role of wildlife in the epidemiology of tuberculosis in Irish cattle.* The four area project, which built on the earlier east Offaly project, provided conclusive evidence of the role of badgers as an important contributor to the epidemiology of tuberculosis in cattle (Griffin *et al.*). Similar results were obtained based on a re-analysis of the east Offaly project (Condon *et al.*). Work was recently completed on the epidemiology of tuberculosis within badger populations, and among badger and cattle populations (three separate studies by Olea-Popelka *et al.*), and further work is underway (Zeeshan *et al.*). A study of the spatial distribution of TB prevalence in badgers recently commenced (McGrath *et al.*). Based on an analysis

of data from the four area project, population control may influence the prevalence of tuberculosis in badger populations (*Corner et al.*). In collaboration with ecologists from University College Cork, we are gaining an improved understanding of the distribution and abundance of badgers in the four area project, both prior to and during disturbance (*Sleeman et al.*).

- *Improving our understanding of risk factors for, and the impact of, herd breakdowns in Ireland.* A series of studies has highlighted risk factors for herd breakdowns, including location, disease history in the herd and herd size (*Olea-Popelka et al.*; *Clegg et al.*). Similar risk factors were identified when considering detection of a TB problem at a factory lesion test (*Olea-Popelka et al.*). Factors associated with the risk of, and animal-level response to, *M. bovis* in Irish cattle are also being considered (*O'Keeffe et al.*). We are also investigating the effect of bovine tuberculosis on milk production in Irish dairy herds (*Boland et al.*), and work is underway to quantify the heritability (both direct and maternal) of susceptibility to tuberculosis (*Berry et al.*).
- *Clarifying the contribution of cattle-to-cattle transmission in the epidemiology of infection in Irish cattle herds.* Residual infection is a contributor to the maintenance of infection in herds (*Olea-Popelka et al.*; *Connolly et al.*). Further studies to clarify the relative importance of various infection sources are currently underway, both generally (*White et al.*) and using data from the four area project (*Clegg et al.*).
- *Contributing information to assist with national decision-making.* The benefits and costs of a pre-movement test have been considered in detail (*Clegg et al.*), as have concerns regarding the relative efficiency of factory surveillance in the disclosure of tuberculous lesions in attested Irish cattle (*Frankena et al.*). Improved methods of measuring progress are currently being considered (*Higgins et al.*). Work is also underway to develop collaborative north-south maps, thereby offering the opportunity to learn from the varied experiences from Ireland and Northern Ireland (*McGrath et al.*). CVERA has also assisted with work to register tuberculin for use within the EU (*Good et al.*). The impact of reactive badger removal on subsequent bovine tuberculosis in cattle herds is currently being investigated, based on data from county Laois (*Olea-Popelka et al.*), and a range of studies underway based on the work of the Wildlife Unit, including ecological (*O'Keeffe et al.*) and epidemiological (*McGrath et al.*) work. The importance of tuberculosis as a zoonotic infection has recently been emphasised (*Doran et al.*).

The TB Diagnostics and Immunology Research Centre

The core activity of the TB Diagnostics Centre is to carry out IFN- γ testing on blood samples from tuberculosis infected herds. The result when reported back to the DVOs are used in conjunction with skin test data and herd history information to devise management schemes to eliminate the infection from the affected herds. Research is also carried out to improve and optimise the performance of the assay, and to evaluate the potential of the test in other situations. e.g., contiguous herd tests, atypical herd breakdowns. When successfully applied, the test provides a means of shortening the period of restriction of infected herds.

The Badger Vaccine Project

The badger vaccine project is a comprehensive programme of research which seeks to develop a vaccine to control tuberculosis in badgers and to break the link of infection to cattle. Fundamental vaccine studies are carried out at a captive facility to test the effect of delivery systems on the generation of protective immunity in badgers. Recent studies have focused on an oral BCG delivery system which we have shown protects badgers from experimental challenge of *M. bovis* inoculated directly into the lung. In collaboration with colleagues at VLA (UK), we are also evaluating a range of immuno-diagnostic tests in naturally TB infected badgers. These tests should dramatically improve our ability to accurately identify infected badgers from a blood sample. The results of these studies will be used in the upcoming vaccine trial where the efficacy of the oral BCG vaccine to protect badgers under conditions of natural transmission will be tested.

Peer-Reviewed Papers

These papers have been written in collaboration with colleagues from Canada, Ireland, New Zealand and the United Kingdom.

The impact of badger removal on the control of tuberculosis in cattle herds in Ireland

Griffin, J.M., Williams, D.H., Kelly, G.E., Clegg, T.A., O'Boyle, I., Collins, J.D. and More, S.J. 2005. The impact of badger removal on the control of tuberculosis in cattle herds in Ireland. *Preventive Veterinary Medicine* 67: 237-266.

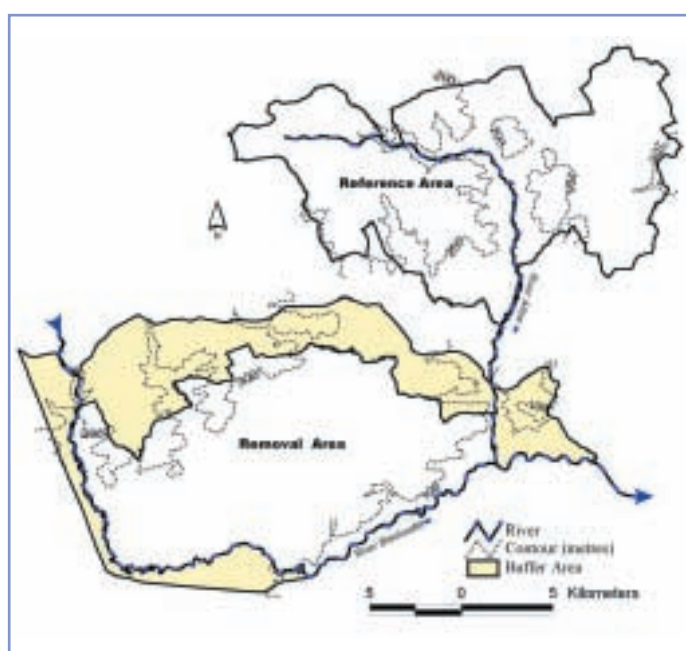
Griffin, J.M., More, S.J., Clegg, T.A., Collins, J.D., O'Boyle, I., Williams, D.H., Kelly, G.E., Costello, E., Sleeman, D.P., O'Shea, F., Duggan, M., Murphy, J. and Lavin, D.P.T., 2005. Tuberculosis in cattle: the results of the four area project. *Irish Veterinary Journal* 58: 629-636.

“Badgers are an important contributor to the epidemiology of tuberculosis in Irish cattle”

There has been a national bovine tuberculosis eradication programme in Ireland since 1954. However, despite intensive measures to eliminate cattle-to-cattle transmission, progress has stalled. Although wildlife were considered an important contributor to the disease in Irish cattle, available evidence to support this assertion was not conclusive. Therefore, the four area project (and the earlier east Offaly project) was conducted to assess the effect of badger removal on the control of tuberculosis in cattle herds in Ireland.

The study was conducted in four matched removal and reference areas, in counties Cork, Donegal, Kilkenny and Monaghan, over five years from September 1997. In the removal areas, a proactive programme of badger removal was conducted 2-3 times each year. In the reference areas, badger removal was entirely reactive; removal operations were conducted in response to severe outbreaks of tuberculosis in cattle herds. The study areas covered approximately 3.9% of the agricultural land area of Ireland.

The results of the study have confirmed that badgers are an important contributor to the epidemiology of tuberculosis in Irish cattle. During the study period, there was a significant difference between the removal and reference areas in all four counties in both the probability of, and the time to, a confirmed herd restriction due to tuberculosis. To illustrate, in the final year of the study, the odds of a herd restriction in the removal as compared to the reference area was 0.25 (95% CI 0.07-0.88) in Cork, 0.04 (0.00-0.27) in Donegal, 0.26 (0.08-0.79) in Kilkenny and 0.43 (0.22-0.84) in Monaghan. The validity of the study has been examined in considerable detail. Efforts to minimise badger-to-cattle transmission in Ireland must be undertaken in association with the current comprehensive control programme, which has effectively minimised opportunities for cattle-to-cattle transmission.



Cork project area

Bovine tuberculosis in badgers in four areas in Ireland: does tuberculosis cluster?

Olea-Popelka, F.J., Griffin, J.M., Collins, J.D., McGrath, G., Martin, S.W., 2003. Bovine tuberculosis in badgers in four areas in Ireland: does tuberculosis cluster? Preventive Veterinary Medicine 59, 103-111.

The purpose of this study was to describe badger distribution, and to investigate whether or not tuberculous badgers had a clustered distribution either within a sett or within a geographic area. We described the distribution of badger populations in 4 different areas in the Republic of Ireland (Donegal, Cork, Kilkenny and Monaghan). The data came from periodic targeted badger-removal and subsequent post-mortem examinations conducted between 1989 and September 1997, and from a formal badger-removal project in the same areas from 1997 through 1999. Records were complete for 2,292 badgers regarding the date of capture, tuberculosis status, geographical area and specific sett from where the badgers were snared. Of 3,187 setts, 2,290 had no badgers recorded against them (i.e. inactive).

The prevalence of tuberculosis differed among areas ranging from 13-29%. Badger populations were highly clustered by sett, and this result was similar over the 4 study areas. The median number of badgers per active sett was 2. Tuberculous badgers also clustered within a sett. The third quartile of tuberculous badgers was 1 per active sett. The prevalence of tuberculous badgers within a sett was not related to the total number of badgers. There was little evidence of spatial clustering with only 1 local cluster of tuberculous setts in each of 3 areas, none in the 4th area. After adjusting for the number of badgers per sett, only 1 area had spatial clusters identified.

“There was little evidence of spatial clustering of tuberculous badger setts in each area”

Breakdown severity during a bovine tuberculosis episode as a predictor of future herd breakdowns in Ireland

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

A retrospective cohort study of Irish cattle herds investigated whether the severity of a herd's bovine-tuberculosis (BTB) breakdown was a predictor of the rate of a future single standard reactor, or multiple standard reactor breakdown in that herd. Data on 10,926 herds not having BTB in 1995 (the 'non-exposed' group) were obtained using a 10% random sample from all herds without BTB in 1995. Data on 6,757 herds that had a new BTB breakdown in 1995 (the 'exposed' group) were obtained and categorized into 5 increasing exposure severity classes based on the total number of standard reactors, to the single intradermal comparative cervical test, detected during the breakdown.

Exposed herds developing BTB, in 1995, were deemed to be free of BTB after they passed a 6 month check test. The first clear test, in 1995, was used as the start of the at-risk period for a non-exposed herd.

In the 5-year period after 1995, 18% of the 'non-exposed' herds had a BTB breakdown, whereas 31% of the exposed herds had a subsequent breakdown. Relative to the hazard for unexposed herds, the risk of the first future singleton standard reactor breakdown, measured using the hazard ratio, ranged from 1.4 for herds with only 1 standard reactor in 1995, up to 1.8 in herds with 4-8 standard reactors during the 1995 episode. When the outcome was 2 or more standard reactors, the hazard ratio ranged from 1.7 for herds with only 1 standard reactor in 1995, up to 2.9 in herds with 8 or more standard reactors during the 1995 episode. The latter hazard varied over time, decreasing to 1.7 after 3 years of risk. Other factors associated with an increased risk of future BTB included a large number of cattle in the herd, a positive history of previous BTB in the herd, and being in an area where the local herd prevalence of BTB was high. The presence of confirmed BTB lesions in reactor cattle was not predictive of the future breakdown rate when the effects of other factors were controlled.

“The severity of a TB breakdown episode, by itself or in conjunction with information on other risk factors, can be useful in predicting the rate of future TB breakdowns in Ireland”

“There is evidence of clustering of *M. bovis* strains at the macro-level (counties) in cattle and badgers, however, there was less evidence of clustering within counties and no clear spatial association between badger and cattle strains”

Spatial relationship between *Mycobacterium bovis* strains in cattle and badgers in four areas in Ireland

Olea-Popelka, F.J., Flynn, O., Costello, E., McGrath, G., Collins, J.D., O'Keeffe, J., Kelton, D.F., Berke, O., Martin, S.W., 2005. *Spatial relationship between Mycobacterium bovis strains in cattle and badgers in four areas in Ireland. Preventive Veterinary Medicine* 71, 57-70.

We investigated whether strains (Restriction Fragment Length Polymorphism, RFLP-types) of *Mycobacterium bovis* isolated from badgers, and from cattle clustered among and within four areas in Ireland. The spatial scan test and nearest neighbor analysis were used as the spatial cluster detection techniques. In addition, for each of the major strains, associations between the distance to badger setts and the "centroid" of the cattle farm were assessed in a logistic model.

Overall, between September 1997 and May 2000, 316 and 287 *M. bovis* samples, from badgers and cattle respectively, were strain-typed. The distribution of strains in badgers, and separately in cattle, differed among areas. Within each of the 4 large areas, badgers and cattle tended to have similar strains; this is consistent with the sharing of *M. bovis* strains within an area. In more detailed within-area analyses, some spatial clusters of *M. bovis* strains were detected, separately, in both cattle and badgers. Almost half of the infected badger setts with a specific strain were located outside of the "detected" clusters. There was no association between the number of infected badgers with a specific *M. bovis* strain within 2 or 5 km distances to cattle herds, and the risk of the same strain in cattle. We speculate about the dynamic nature of badger movements, as an explanation for the absence of more clusters of most of the strains of *M. bovis* isolated from badgers, and its impact on trying to study transmission of *M. bovis* between cattle and badger.

“The electronic nose is an alternative to conventional methods of TB diagnosis, and it offers considerable potential as a sensitive, rapid, and cost-effective means of diagnosing *M. bovis* infection in cattle and badgers”

Use of an electronic nose to diagnose *Mycobacterium bovis* infection in badgers and cattle

Fend, R., Geddes, R., Lesellier, S., Vordermeier, H.M., Corner, L.A., Gormley, Costello, E., Hewinson, R.G., Marlin, D. J., Woodman, A.C., Chambers, M.A., 2005. *Use of an electronic nose to diagnose Mycobacterium bovis infection in badgers and cattle. Journal of Clinical Microbiology* 43, 1745-1751.

The accurate and reliable diagnosis of infection with *Mycobacterium bovis* is a critical component of tuberculosis control. New *in vitro* diagnostics are required to allow disease surveillance in badger populations, as well as to support a future vaccine strategy. In this study, we have investigated the potential of an 'electronic nose' (EN) to diagnose infection of cattle and badgers with *M. bovis* by using a serum sample. The 'electronic nose' is the colloquial name for an instrument made up of chemical sensors combined with a pattern recognition system. The study demonstrated that EN technology could be used for the diagnosis of tuberculosis in both cattle and badgers and is the first report of the application of EN sensing to serum.

Molecular detection of *Mycobacterium bovis* and *Mycobacterium bovis* BCG (Pasteur) in soil

Young, J.S., Gormley, E., Wellington, E. M. H., 2005. Molecular detection of *Mycobacterium bovis* and *Mycobacterium bovis* BCG (Pasteur) in soil. *Applied and Environmental Microbiology* 71, 1946-1952.

Wildlife reservoirs of *Mycobacterium bovis* are of importance due to the significant number of bovine tuberculosis breakdowns in cattle herds in both Ireland and in the United Kingdom. The badger (*Meles meles*) has been implicated, but it is unclear how the disease is transmitted to cattle from badgers. Few studies have addressed dissemination and persistence of environmental *M. bovis*. Survival of up to 6 weeks for *M. bovis* cells inoculated into soil and feces, detected by traditional selective cultivation methods has been reported. However, cultivation techniques for monitoring *M. bovis* and other mycobacteria in soil are impeded by the slow growth rates of *M. bovis* and the need for prolonged incubation of highly selective agars. In this study, we present the first report of the use of analysis of community DNA with specific PCR primers targeting both antigen genes and the *M. bovis* 16S rRNA gene to demonstrate the long-term survival of *M. bovis* in environmental samples.

“This study provides clear evidence that *M. bovis* can persist in the farm environment outside of its hosts and that climatic factors influence survival rates”

Conjunctival vaccination of the brushtail possum (*Trichosurus vulpecula*) with bacille Calmette-Guérin

Corner, L.A.L., Buddle, B.M., 2005. Conjunctival vaccination of the brushtail possum (*Trichosurus vulpecula*) with bacille Calmette-Guérin. *New Zealand Veterinary Journal* 53, 133-136.

In New Zealand the brushtail possum (*Trichosurus vulpecula*) is the principle wild life reservoir of bovine tuberculosis. BCG vaccine has been found to induce significant level of protective immunity. The number of routes by which BCG vaccine can be administered to free-ranging wild animals is limited. It was found that conjunctival vaccination with BCG induced a significant level of protective immunity against experimental *M. bovis* challenge.

“Conjunctival vaccination of possums with BCG induced significant protection equivalent to other routes of administration”

“The changed behaviour of possums in the terminal stages of tuberculosis increases the risk of transmission to cattle and deer”

Ranging behaviour and duration of survival of wild brushtail possums (*Trichosurus vulpecula*) infected with *Mycobacterium bovis*

Norton, S., Corner, L.A.L., Morris, R.S., 2005. Ranging behaviour and duration of survival of wild brushtail possums (*Trichosurus vulpecula*) infected with *Mycobacterium bovis*. *New Zealand Veterinary Journal* 53, 293-300.

In New Zealand the brushtail possum (*Trichosurus vulpecula*) is the principle wild life reservoir of bovine tuberculosis (*M. bovis* infection). Using radio-telemetry and cage trapping, the behaviour of, and duration of the clinical phase of infection was studied in 14 natural-infected and 8 experimentally-infected possums. The clinical phase of infection was found to be very short, 3.4 months. In the pre-terminal stages the possum behaviour remained within normal limits. Only in the last 3 weeks of the terminally ill phase of infection when progressive debilitation and weakness became apparent did the possums show changed behaviours. The changes (activity during daylight, loss of avoidance behaviour and weakness) would all have increased the risk of exposure to cattle and deer.

“A delay in processing of the blood samples may potentially result in *M. bovis* infected animals remaining undetected in a herd”

The effect of the tuberculin test and the consequences of a delay in blood culture on the sensitivity of a gamma-interferon assay for the detection of *Mycobacterium bovis* infection in cattle

Gormley, E., Doyle, M.B., McGill, K., Costello, E., Good, M., Collins, J.D., 2004. The effect of the tuberculin test and the consequences of a delay in blood culture on the sensitivity of a gamma-interferon assay for the detection of *Mycobacterium bovis* infection in cattle. *Veterinary Immunology and Immunopathology* 102, 413-20.

The tuberculin test, in its various forms, has performed well as a mass screening test for tuberculosis in cattle. However, due to a lack of absolute sensitivity, an assay system for IFN- γ has been developed in Australia in order to enhance the success rate of diagnosis of infection. Questions have been asked as to the impact of the skin test and a delay in processing of blood on the reliability of the assay. In this study we demonstrated that a delay in processing of the blood samples from cattle subjected to routine surveillance could significantly impact on the outcome of the IFN- γ assay resulting in a change of the IFN- γ status of the animals.

“The epidemiology and pathogenesis of Johne's disease in cattle needs to be clarified”

The respiratory route as a hypothetical aerosol transmission pathway for *Mycobacterium avium* subspecies *paratuberculosis* infection in cattle

Corner, L.A.L., Pfeiffer, D.U., Abbott, K.A., 2004. The respiratory route as a hypothetical aerosol transmission pathway for *Mycobacterium paratuberculosis* infection in cattle. *Australian Veterinary Journal* 82, 170-173.

The epidemiology of Johne's Disease in cattle is not fully understood and evidence from experimental infection studies does not fully support the commonly held belief that the pathway for transmission is the faecal-oral route. The paper presents evidence and arguments in favour of a respiratory route of transmission involving infectious aerosols.

Published Reports, Conference Papers and Posters, Industry Papers

4th International Conference on *Mycobacterium bovis*

Over 300 scientists from 35 countries attended the 4th International Conference on *Mycobacterium bovis* at Dublin Castle during 22-26 August 2005, highlighting the ongoing importance of bovine tuberculosis in many countries. In common with previous conferences in this series (Dublin in 1991, Dunedin in 1995 and Cambridge in 2000), the meeting facilitated the sharing of knowledge and ideas among policy-makers, stakeholders and research scientists with the aim of addressing current constraints to the control and eradication of tuberculosis in livestock. The conference was organised by CVERA, under the auspices of the Department of Agriculture & Food (DAF), Dublin and the Department of Agriculture and Rural Development (DARDNI), Belfast

The 28 plenary papers and the workshop reports have been compiled as a special edition of *Veterinary Microbiology* (volume 112, issues 2-4, 25 February 2006), under the guest editorship of S.J. More, J.D. Collins, E. Gormley, M. Good, R.A. Skuce and J.M. Pollock. Papers from staff of CVERA, the TB Diagnostic and Immunology Research Centre and the Badger Vaccine Project include:

- More, S.J., Collins, J.D., Gormley, E., Good, M., Skuce, R.A. and Pollock, J.M. Preface.
- More, S.J. and Good, M. The tuberculosis eradication programme in Ireland: a review of scientific and policy advances since 1988.
- Collins, J.D. Strategic planning for the future.
- Gormley, E., Doyle, M.B., Fitzsimons, T., McGill, K. and J. D. Collins. Diagnosis of *Mycobacterium bovis* infection in cattle by use of the gamma-interferon (Bovigam®) assay.
- Corner, L.A.L. The role of wild animal populations in the epidemiology of tuberculosis in domestic animals: how to assess the risk.
- Workshop reports, including:
 - Wildlife vaccination: Policy and strategy
 - Managing national bovine tuberculosis eradication programmes
 - Science-policy interface: Revisiting diagnosis
 - Science-policy interface: Applying molecular epidemiology to problem-solving
 - Science-policy interface: Wildlife vaccination (further considerations)



“The conference was attended by over 300 scientists and veterinarians from 35 countries, highlighting the ongoing importance of bovine tuberculosis in many countries”



“Transmission of infection from wildlife to cattle is a key constraint to the eradication of bovine tuberculosis from Ireland”

A critical review of progress in the national Irish control programme

Centre for Veterinary Epidemiology and Risk Analysis, 2004. The impact of badger removal on the control of tuberculosis in cattle herds in Ireland. A report to the Minister of Agriculture and Food, University College Dublin, Dublin.

Griffin, J.M, Clegg, T.A., Kelly, G.E., Williams, D.H., O'Boyle, I., Collins, J.D. and More, S.J. 2005. The impact of badger removal on the control of tuberculosis in cattle herds in Ireland. Society for Veterinary Epidemiology and Preventive Medicine, 30th March - 1st April 2005, Nairn, Inverness, Scotland, p42-54.

More, S.J., 2005. Towards eradication of bovine tuberculosis in Ireland: a critical review of progress. Society for Veterinary Epidemiology and Preventive Medicine, 30th March - 1st April 2005, Nairn, Inverness, Scotland, p13-23 (plenary talk).

More, S.J., 2005. Eradication of bovine tuberculosis. Veterinary Today, the Journal of the Veterinary Profession in Northern Ireland. Summer 2005: 81-84.

Results from the four area project and related aspects of the broader research programme, have been presented within the international peer-reviewed literature. In addition, this information has been presented to a broader audience, including the Minister of Agriculture & Food, veterinary epidemiologists throughout Europe, and to veterinarians in Northern Ireland.

Other conference presentations and posters

*Corner, L.A.L, Gormley, E., O'Meara, D., Costello, E. 2005. Tuberculosis in badgers: True prevalence, diagnostic methods and epidemiology. Vaccination of badgers against tuberculosis: the optimal dose of *Mycobacterium bovis* required for challenge. 4th International Conference on *Mycobacterium bovis*, 22-26 August 2005, Dublin (poster).*

Corner L.A.L., Lesellier S., Costello E., Sleeman P. and Gormley E. Development of a wildlife vaccination strategy for eradication of tuberculosis in cattle in Ireland. Association of Veterinary Teachers and Research Workers, Scarborough, 5-7 April 2004 (oral presentation).

*Frankena, K., van Grevenhof, I., Martin, S.W., O'Keeffe, J., Costello, E. White, P. and More, S.J., 2005. Quantification of the relative efficiency of factory surveillance in the disclosure of tuberculous lesions in attested Irish cattle. 4th International Conference on *Mycobacterium bovis*, 22-26 August 2005, Dublin (oral presentation).*

*Gormley, E., Doyle, M.B., McGill, K., Fitzsimons, T., Good, M., Collins, J.D. 2005. Diagnosis of *M. bovis* infection in Ireland: The role of the Bovigam IFN- γ test. 4th International Conference on *Mycobacterium bovis*, 22-26 August 2005, Dublin (poster).*

*Lesellier S., Corner L.A.L., Sleeman P., Chambers, M.A., Hewinson, R.G., Costello E. and Gormley E. Antigen recognition in badgers experimentally infected with *Mycobacterium bovis*. Association of Veterinary Teachers and Research Workers, Scarborough, 5-7 April 2004 (oral presentation).*

*Lesellier, L., Corner, L.A. Sleeman, D.P.S., Chambers, M.A., Hewinson, R.G., Costello, E., Gormley, E. 2005. Development of a wildlife vaccine against tuberculosis: Immuno-diagnosis of disease in experimentally infected badgers. 4th International Conference on *Mycobacterium bovis*, 22-26 August 2005, Dublin (oral presentation).*

Lesellier, L., Corner, L.A., Sleeman, D.P.S., Chambers, M.A., Hewinson, R.G., Costello, E., Gormley, E. 2005. Vaccination of badgers against tuberculosis: the optimal dose of *Mycobacterium bovis* required for challenge. 4th International Conference on *Myobacterium bovis*, 22-26 August 2005, Dublin (poster).

Lesellier, L., Corner, L.A., Sleeman, D.P.S., Chambers, M.A., Hewinson, R.G., Costello, E., Gormley, E. 2004. Antigen recognition in badgers experimentally infected with *Mycobacterium bovis*. 7th International Veterinary Immunology Symposium. 25-30 July 2004, Quebec (oral presentation / poster).

O'Keeffe, J. and White, P., 2005. A review of aspects of the epidemiology of TB in the Irish cattle herd. Society for Veterinary Epidemiology and Preventive Medicine, 30th March - 1st April 2005, Nairn, Inverness, Scotland (poster).

O'Keeffe, J. and McGrath, G., 2005. A managed reduction of the badger population in the Republic of Ireland as medium-term strategy toward eradication of tuberculosis in cattle. Society for Veterinary Epidemiology and Preventive Medicine, 30th March - 1st April 2005, Nairn, Inverness, Scotland (poster).

Olea-Popelka F.J., Phelan, J., White P.W., Mc.Grath, G., Collins J.D., O'Keeffe, J., Duggan, M., Collins, D. M., Kelton D.F., Berke, O., More, S. and Martin S.W. 2006. Quantifying badger exposure and the risk of bovine tuberculosis for cattle herds in county Kilkenny, Ireland. Graduate Student Appreciation Day, University of Guelph, April 2006 (oral presentation).

Olea-Popelka F.J., Phelan, J., White P.W., Mc.Grath, G., Collins J.D., O'Keeffe, J., Duggan, M., Collins, D. M., Kelton D.F., Berke, O., More, S. and Martin S.W. 2006. Quantifying badger exposure and the risk of bovine tuberculosis for cattle herds in county Kilkenny, Ireland. Society for Veterinary Epidemiology and Preventive Medicine, 30th March - 1st April 2005, Nairn, Inverness, Scotland (poster).

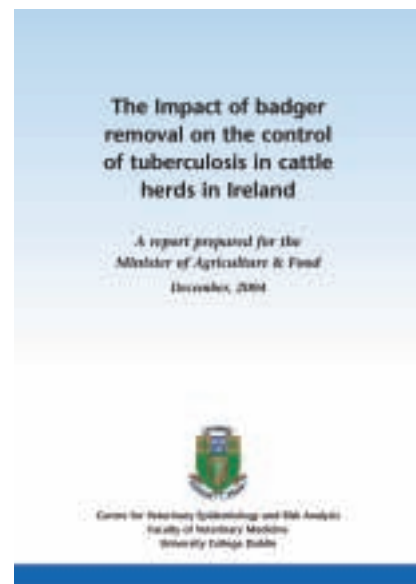
Olea-Popelka F.J., Flynn O., Costello E., McGrath G., Collins J. D., O'Keeffe J., Kelton D.F., Berke O. and Martin S.W. Spatial distribution of *Mycobacterium bovis* strains in cattle and badgers in four areas in Ireland. 4th International Conference on *Myobacterium bovis*, 22-26 August 2005, Dublin (oral presentation).

Olea-Popelka F.J., Flynn O., Costello E., McGrath G., Collins J. D., O'Keeffe J., Kelton D.F., Berke O. and Martin S.W. Spatial distribution of *Mycobacterium bovis* strains in cattle and badgers in four areas in Ireland. GISvet Conference. University of Guelph, June 2004 (poster).

Olea-Popelka F. J., Griffin J.M., Collins J.D., McGrath G. and S.W. Martin. Bovine Tuberculosis in Badgers in the Republic of Ireland: Clustering ? 10th International Symposium for Veterinary Epidemiology and Economics (ISVEE), Viña del Mar, Nov. 2003 Chile (oral presentation).

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Work in progress

“When the IFN- γ assay is used in parallel with the tuberculin test, it is capable of identifying infected cattle which might otherwise not be detected by the skin test until later, if at all”

TB Diagnostics & Immunology Research Centre

Principal investigators: Mairead Doyle, Kevina McGill, Tara Fitzsimons, Dan Collins (UCD), Margaret Good (DAF), Eamonn Gormley (UCD)

The TB Diagnostics & Immunology Research Centre is based at UCD and carries out bovine tuberculosis blood testing and research for the Dept. of Agriculture and Food. Blood samples are submitted to the laboratory from qualifying herds and following a two stage IFN- γ ELISA assay, the results are returned to the referring District Veterinary Office within a few days. In 2005 a total of 8,400 samples were submitted for testing from infected herds. The majority of samples were submitted from herds with a recent history of tuberculosis. When the IFN- γ assay is used in parallel with the tuberculin test, it is capable of identifying infected cattle which might otherwise not be detected by the skin test until later, if at all. The early detection and removal of these animals reduces the risk that they will become a source of infection for other cattle. When targeted in herds of high Tb prevalence, the benefits to the herd owner directly concerned can be considerable as the IFN- γ assay provides a means of shortening the period of restriction for such herds. Samples are also submitted from 'atypical' herds containing skin test reactors but no other evidence of infection (e.g. presence of lesions at slaughter). The test is also currently being evaluated for its potential use in other specific conditions e.g., identifying infected herds contiguous to a known TB infected herd.

As with the tuberculin skin test, the lack of absolute sensitivity and specificity of the IFN- γ assay test has led to several studies being carried out to determine factors that critically influence the reliability of the assay. However, it is unlikely that the IFN- γ assay will replace the tuberculin skin test as a routine screening test because of the lower specificity of the IFN- γ test. We are particularly interested in host responses that might impact on IFN- γ production in infected cattle and how these might be exploited to enhance the usefulness of the test. We have recently shown that the test is optimal when the blood is assayed on the same day as sample collection, which means the samples must reach the laboratory for processing within 8 hours. Delaying the test beyond this can result in a significant loss of sensitivity with the potential for infected animals to remain undetected. The test is also currently being evaluated using different sources of tuberculin to determine if the sensitivity of the test varies according to the tuberculin used. Recognition that the purpose of the assay is to identify high-risk animals that are potentially infectious for other cattle can generate confidence in herd-owners that rational decisions can be made based on sound scientific principles, and that effective schemes can be devised to make more rapid progress in the elimination of the infection from affected herds.

Development of a badger vaccine against tuberculosis

Principal investigators: Eamonn Gormley, Leigh Corner, Denise Murphy, Sandrine Lesellier (UCD) and Eamon Costello (DAF)

In Ireland, the badger acts as a maintenance host for *Mycobacterium bovis* and contributes to the spread and persistence of tuberculosis in domestic livestock. While continued tuberculin testing will serve to maintain tuberculosis in cattle at a low level, there is a consensus that eradication of *M. bovis* infection in badgers and, consequently, in cattle, will not be solved without a vaccine.

The development of a vaccine against tuberculosis for use in the badger population

was initiated by the Dept. of Agriculture & Food in 1998. Drawing on the latest national and international developments in immunology and vaccine research, the badger vaccine group based at University College Dublin, in conjunction with colleagues in Dept. of Zoology (University College Cork) and Central Veterinary Research Laboratories (Abbotstown), have embarked on an ambitious program to develop a vaccine to protect badgers against tuberculosis. The vaccine, when available, should serve the purpose of preventing disease in the badger, thereby breaking the chain of infection between badgers and cattle. In parallel with the vaccine program and in collaboration with DEFRA-UK scientists based at VLA Weybridge, we have also helped to develop, and are currently testing, a range of novel diagnostic tests for the rapid diagnosis of tuberculosis in live badgers.

The sole existing vaccine for tuberculosis, the attenuated *M. bovis* strain Calmette-Guérin (BCG), was developed in the early 1900's from a virulent *M. bovis* isolate and has since been widely used to control tuberculosis in humans, with varying degrees of success. Vaccination studies carried out in New Zealand with cattle, deer and brushtail possums have demonstrated that the BCG vaccine, when delivered under appropriate conditions, can generate significant protective immunity against experimental challenge with virulent *M. bovis*. The BCG was also shown to induce significant levels of protective immunity in wild possums.

In the first vaccine study carried on Irish badgers, two groups of five badgers were vaccinated either by subcutaneous injection or intranasal/conjunctival instillation of the BCG vaccine. Also included was a non-vaccinated control group of five badgers. At 12 weeks post-vaccination, all 15 badgers were challenged with virulent *M. bovis* by endobronchial inoculation. At 12 weeks post-challenge they were examined post mortem. In each of the vaccine groups, there was a significant reduction in the severity of disease when compared with the control group, where all five badgers had severe lesions of tuberculosis. From these observations and additional immunological data analysis, we concluded that the vaccine produced a significant level of protection against tuberculosis.

In a follow up study we vaccinated a group of badgers by the oral route, with the BCG encapsulated in a lipid matrix to minimize degradation of the vaccine as it transits through the GI tract. This specialized formulation has been developed by a collaborating group headed by Dr Frank Aldwell at the University of Otago in New Zealand. Badgers vaccinated with this formulation also showed reduced levels of disease after challenge when compared with the non-vaccinated controls. We are currently evaluating data from a separate study that set out to examine the duration of the immune effect in vaccinated badgers. The results to date from all of our vaccine studies have established as proof of principle that oral vaccination is a feasible option for delivery of an effective vaccine to badgers.

Preparations are already underway to conduct a field vaccination trial in order to assess the impact of vaccination on badger-to-badger transmission of tuberculosis in a natural environment. This field trial will involve vaccinating several hundred badgers over 3-4 years with continuous monitoring of the population to assess the impact of the vaccine on the incidence of disease in the vaccinated and non-vaccinated control populations. As a prerequisite to this study we are validating the recently developed blood tests on 200 badgers removed from selected areas of the country by the DAF Wildlife Unit. These badgers have been subjected to detailed necropsy followed by bacterial culture from a comprehensive range of tissues and lymph nodes. When available, the culture results will be compared with those obtained using the blood tests. In conjunction with a parallel study being conducted in the UK, the combined data set will provide an accurate determination of the sensitivity of the blood tests. These tests will constitute a critical component of the upcoming vaccine field trial.

“The vaccine, when available, should serve the purpose of preventing disease in the badger, thereby breaking the chain of infection between badgers and cattle”

“Although cattle-to-cattle transmission is now considered of lesser importance as a result of the national control programme, additional strategies may help to limit this further. Pre-movement testing is one such option”

The benefit-cost of a pre-movement test in Ireland

Principal investigators: Tracy Clegg, Simon More and Isabella Higgins (CVERA)

Recent work has highlighted the importance of transmission from wildlife in the epidemiology of bovine tuberculosis in Ireland, and steps are now being taken to address this. Although cattle-to-cattle transmission is considered of lesser importance as a result of the national control programme, additional strategies may help to limit this further. Pre-movement testing is one such option.

In this study, we are seeking to determine the proportion of herd breakdowns that could reasonably be attributed to the introduction of an infected animal, to describe events between de-restriction and the next full herd test, to estimate the proportion of animals infected at the time of de-restriction, to identify high-risk movements (those most likely to involve infected animals), to identify the optimum time for testing an animal following de-restriction, and to determine the benefit-cost of a pre-movement test in an Irish context.

This study was conducted in two parts, including a retrospective investigation of all reactors or animals identified as having lesions, at a breakdown, and a prospective investigation was conducted of animal movements once a herd that previously had a tuberculous breakdown was subsequently declared disease-free. Three national databases are being used during this work, including databases associated with the Cattle Movement Monitoring System, the Animal Health Computer System and the factory lesion recording system. A range of methodologies are being used, including the development of logistic regression models to identify herds with the highest risk of selling an infected animal and animals that are at the highest risk of subsequently becoming a reactor.

“Culling may influence the prevalence of tuberculosis in badger populations”

The effect of varying levels of population control on the prevalence of tuberculosis in badgers in Ireland

Principal investigators: Leigh Corner (UCD), Tracy Clegg and Simon More (CVERA)

The effect of differing culling regimes on the prevalence of tuberculosis in badger populations had not been formally examined. During the four area study, different culling regimes were employed in the removal, buffer and reference areas. All badgers culled during the study and badgers killed in road traffic accidents were subjected to post mortem examination for *M. bovis* infection. These data are being examined to better understand the effect of different levels of population control on the prevalence of tuberculosis in badgers.

Quantifying badger exposure and the risk of bovine tuberculosis for cattle herds in County Kilkenny, Ireland: A case control study

Principal investigators: Francisco Olea-Popelka, Wayne Martin (University of Guelph) and James O'Keeffe (CVERA)

This is a matched case control study in which the specific location of cattle within each farm, and the length of time that cattle spent in each farm field during the grazing season, and in the barn-yard during winter, was used. These data were used to build an 'exposure coefficient' that allowed the quantification of the amount of badger exposure that cattle encounter both on pasture and in the barn. The objectives of the study were to quantify the levels of badger exposure for cattle, and to test the hypothesis that increased exposure does not increase the risk of bovine tuberculosis in a herd. We used data collected during 1996 to 1999 in County Kilkenny. During the 4-year study period, 96 tuberculosis breakdowns occurred. The control herds were selected using incidence density sampling at the same time as the case herd breakdown. In total, 543 badgers were removed during the study period, and of these, 96 badgers were classified as tuberculosis positive. There was a significantly increased risk ($RR=2.1$) of finding tuberculous badgers when the badger removal operations were conducted in areas within 2 km of farms infected with bovine tuberculosis rather than elsewhere in the study area. A significant association between case herds and the odds of having a higher badger sett 'exposure coefficient' during 1996-1998 was found. However, we found no significant association between case herds and the odds of having a higher 'exposure coefficient' that considered the number of badgers or tuberculous badgers during September 1997 to December 1999.

“An association was found between TB infected herds and proximity to badger setts. However, no association was found with proximity to infected badger setts”

A case study of bovine tuberculosis in a group of herds in County Donegal, Ireland

Principal investigators: Francisco Olea-Popelka, Wayne Martin (University of Guelph) and James O'Keeffe (CVERA)

We performed a descriptive analysis to investigate the potential risk factors that might have contributed to the increased incidence of bovine tuberculosis (BTB) herd-breakdowns in the reference area of Co. Donegal during the fifth year of the four-area project (FAP). Seventy two different herds were restricted for BTB during the FAP; 10 of these herds were restricted twice, resulting in a total of 82 BTB breakdowns.

During the first four years of the FAP, the number of BTB herd breakdowns in the area varied from a lowest of 9 to a maximum of 18 per year, and were geographically dispersed. In the fifth year of the study a considerable increase in the number of BTB breakdowns ($n=32$) was observed, and there was a spatial "cluster" of infected herds in the eastern part of the study area.

The increased number of BTB breakdowns during the fifth year most likely occurred because of the recrudescence of infection, herd-to-herd transmission, and to a lesser extent following purchase of infected cattle. Infected badgers remain as a possible but less likely source of infection, especially as an explanation for the cluster of infected herds.

Our analysis supports the hypothesis that BTB in herds is a problem that cannot be addressed successfully by dedicating efforts to the elimination of single risk factors. Neither is it a problem that needs to be investigated only at the herd level, but rather at the area level, including groups of contiguous herds.

“Bovine tuberculosis in herds is a problem that cannot be addressed successfully by dedicating our efforts to the elimination of single risk factors”

“Our findings support the widely accepted view that TB is a slowly progressive, chronic-infectious disease transmissible from cattle to cattle”

Risk factors for tuberculosis in clear cattle herds that disclosed an animal with a tuberculosis lesion at slaughter during 2003 in Ireland

Principal investigators: Francisco Olea-Popelka, Wayne Martin (University of Guelph) and James O'Keeffe (CVERA)

As part of the ongoing screening processes for bovine tuberculosis (BTB) in Ireland, all animals are examined at the time of slaughter for evidence of disease, including BTB. If BTB is confirmed, either by histopathology or culture, a herd-test using the Single Intradermal Comparative Tuberculin Test (SICTT) is scheduled for the herd that sold the animal to the factory; this test is known as a 'factory lesion' herd test.

The objective of our study was to identify risk factors for the herds that have a BTB problem (SICTT-positive result) at the factory-lesion test.

1,713 BTB-clear seller herds with 1 animal with a confirmed BTB lesion at slaughter during 2003 were identified. At the subsequent herd test, 338 (19.7%) were classified as BTB-problem herds.

Our analysis showed that two factors seem to be of paramount importance; namely, the time that the index lesioned animal spent in the herd, and the presence (or otherwise) of a BTB-lesioned animal in a previous BTB episode. The risk posed by the presence of a lesioned animals during this episode is related to the length of time since this episode occurred. Other factors such as herd size and the interval between herd tests also increased the risk of BTB problems.

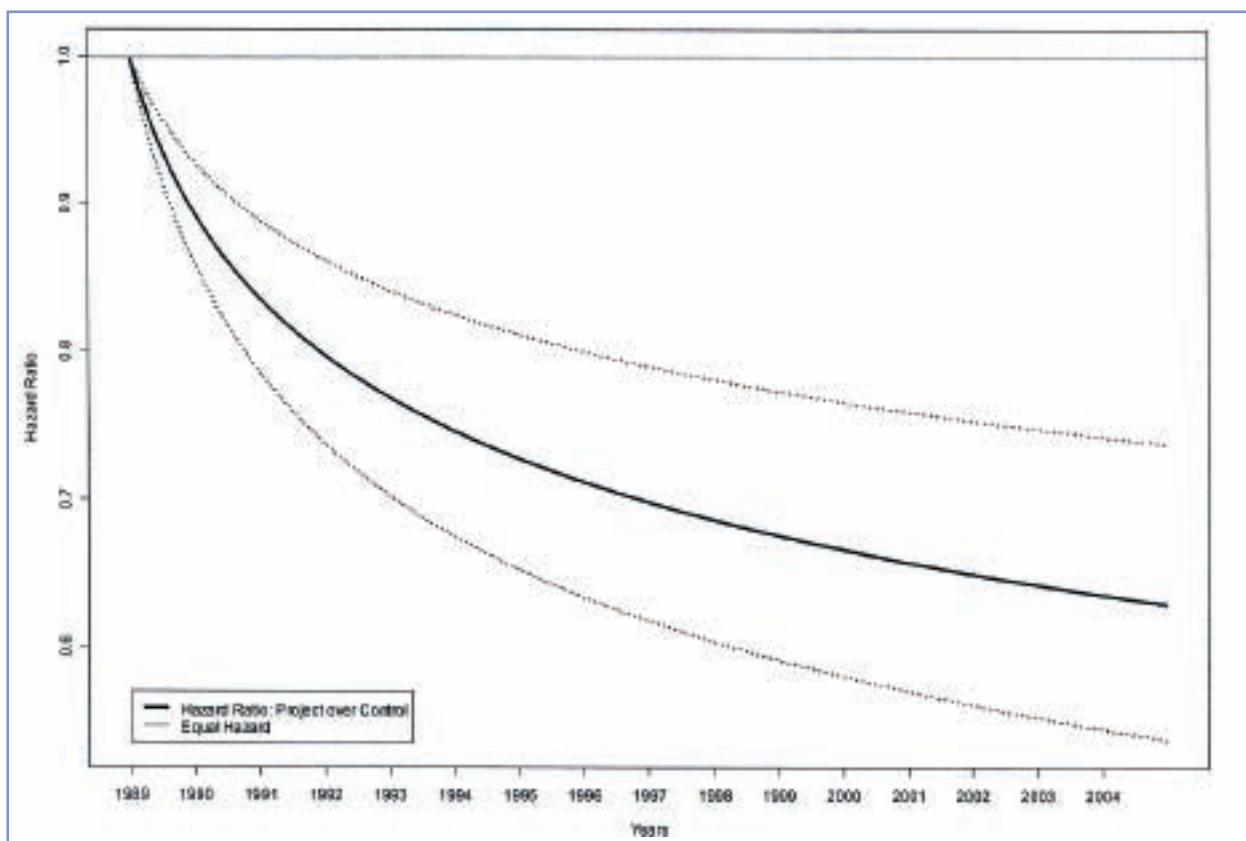
Although there is no precise way of predicting the temporal course of BTB within an animal, we accept that after infection risk is increased with increasing time of the animal in the risk herd.

A long term study of the impact of proactive badger removal on herd restrictions due to bovine tuberculosis in east Offaly, 1989 – 2004

Principal investigators: Joe Condon (Queen's University) and Gabrielle Kelly (UCD)

The east Offaly and four area projects provide conclusive evidence of the link between the removal of badgers and the occurrence of bovine TB on Irish farms. It is now some years since the formal conclusion of the east Offaly project, and data are now available to assess the longer-term effects of proactive badger removal. Therefore, this study is seeking to assess the impact of badger removal on the disclosure of cattle tuberculosis, both during and following a formal period of proactive badger removal. A formal programme of badger removal was conducted in east Offaly from 1989-1995. This study is re-examining these data, but also subsequent data until the end of 2004. The data are analysed using survival analysis methods; specifically, a Cox regression model with the Anderson & Gill method for modelling multiple events. The model has project/control, herd size and previous history as time dependent covariates. Because the effect of being in the project area can change over time, it is being modelled using both polynomials and psplines.

“This study is investigating the long term impact of proactive badger removal on herd restrictions due to bovine tuberculosis”



Estimated hazard ratio (with 95% confidence interval) of a confirmed restriction in the project as compared to the control area of the East Offaly Project during 1989 - 2004

“By focusing on the minority of herds having extended restriction periods, this analysis will consider the possibilities for defining ‘chronic herds’”

Quantification of bovine tuberculosis transmission in Irish cattle herds and optimising the eradication program (Descriptive statistics, 1995-2004)

Principal investigators: Paul White (CVERA) and Klaas Frankena (Wageningen University)

The present range of descriptive statistics produced by Dept. of Agriculture and Food (DAF) has a within-year focus, with lesser emphasis on between-year comparisons. Existing data tells one the numbers of herds tested and the number failed, but does not give any information relating to how many herds that were positive in any given year that had a previous tuberculosis episode.

Given that tuberculosis is a highly clustered disease, focusing on the herd as the unit of interest is to ignore the wider epidemiology of the spatial event, which might be more appropriate as the focus of interest. Preliminary work have shown that close to 50% of herds have no episode of tuberculosis, suggesting that the disease is cycling to a large degree among a core group of affected herds.

The pattern of reactor disclosure varies, with most herds regaining their trading status after a positive index test and 2 clear reactor retests. This study will further describe the range of patterns of reactor disclosure over time. By focusing on the minority of herds having extended restriction periods, this analysis will consider the possibilities for defining ‘chronic herds’.

It is proposed to classify herds using the herd classification system currently employed by the Dept. of Agriculture and Food to identify herds with a recent history of tuberculosis. The national herd currently numbers circa 130,000 and 8-10,000 herds experience a tuberculosis episode in any year, it is important to know how many of these are new episodes and how many are reoccurrences.

These descriptive analyses will also seek to define clusters of tuberculosis episodes that have occurred within the data set. Using spatial data to identify herds within a 25-meter search radius, it will be possible to establish the first herd identified with tuberculosis in a local area, and to assign each subsequent breakdown herd within the 25-meter radius as part of that cluster. Testing data from each year will be reorganised at the level of the cluster, and descriptive measurements relating to these clusters will be proposed and evaluated. Further, by comparing spatial patterns over time, we will be able to quantify the degree to which clusters overlap, and whether some areas have a greater number of overlapping clusters. Chronic areas will be identified, using objective criteria.

Quantifying the relative efficiency of factory surveillance in the disclosure of tuberculous lesions in attested Irish cattle

Principal investigators: Klaas Frankena, Ilsa van Grevenhof (Wageningen University), Paul White, James O'Keeffe and Simon More (CVERA)

The detection of gross lesions in attested cattle at slaughter, so-called factory surveillance, is important to the detection of infected herds within the national tuberculosis eradication programme in Ireland. The objective of the study was to determine the comparative efficiency of lesion detection among attested cattle in 42 Irish factories, based on lesion submission and bovine tuberculosis (BTB) confirmation. National databases on animal slaughter (years 2002 and 2003), BTB testing and laboratory confirmation of suspected lesions were available. Factories were ranked according to their submission ratio while adjusted for the risk profile of the animals presented for slaughter. This profile included 5 risk factors related to animal characteristics (age and gender), the herd of origin (BTB test history of the herd, BTB prevalence of surrounding herds) and season of slaughter. Preliminary results suggest that the risk-factor adjusted proportion of slaughtered animals that were submitted to further laboratory confirmation varied between factories (Figure 1). Adjustment for risk factors had little effect on the proportion submitted and on the ranking of factories. The risk-factor adjusted proportion of submitted animals that were subsequently tested positive also varied between factories (Figure 2). Further research is required to shed light on the origin of this between-factory variation.

“The relative efficiency of surveillance (the detection of gross lesions in attested cattle at slaughter) varied widely between factories”

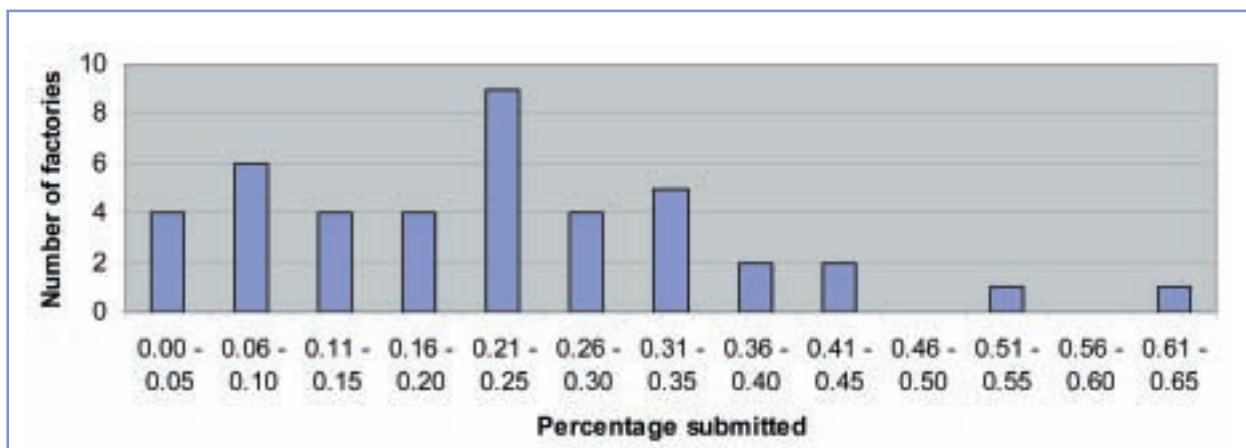


Figure 1: Frequency distribution of adjusted submission rates

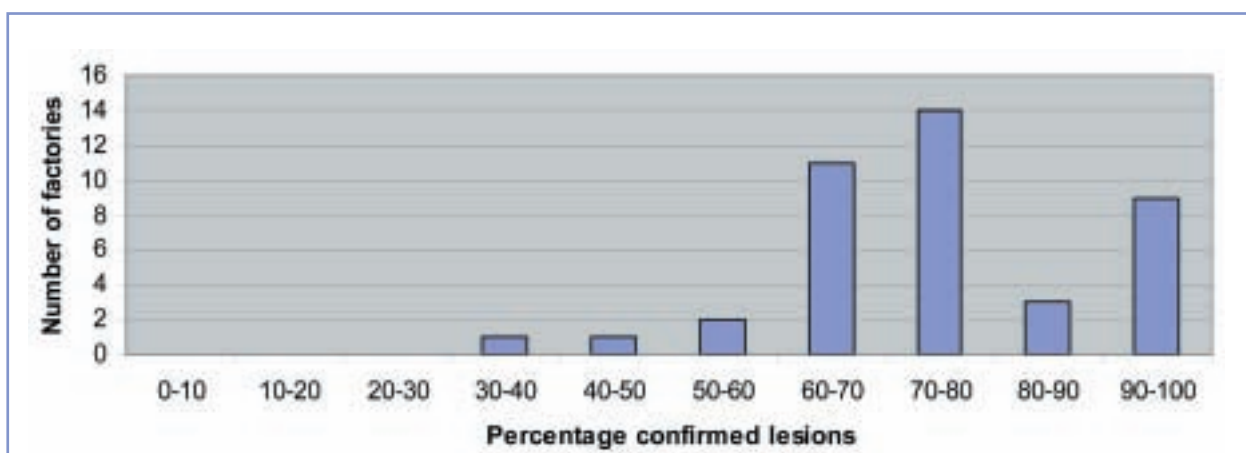


Figure 2: Frequency distribution of adjusted confirmed lesion rates

“A series of studies is being undertaken in support of BCG vaccination of badgers as a control tool in the national programme”

Studies to support BCG vaccination of badgers as a control tool in the national programme

Principal investigators: Denise Murphy, Leigh Corner, Eamonn Gormley (UCD), Eamonn Costello (DAF)

A series of studies are being undertaken in support of BCG vaccination of badgers as a control tool in the national programme, including:

- A study is currently underway to determine the duration of protection in badgers from a single oral dose of BCG. The question is an important one. In the field we need to know how frequently revaccination will be required to provide adequate protection. Badgers were challenged at 3, 6 and 12 months after vaccination. Protection will be measured using immunological and pathological parameters.
- Several different immunodiagnostic assays for the diagnosis of TB in badgers have been developed. The sensitivity of these assays to detect infection in naturally infected animals has not been established. Studies are being carried out on blood from 215 badgers and compared to the isolation of *M. bovis*, the definition of infection.
- A study of the pathogenesis of infection in naturally infected badgers is being conducted. This study aims to determine the significance of the lungs as the primary site of *M. bovis* infection in naturally infected badgers. Samples from 215 culled badgers have been collected.

“Knowledge of factors that influence an animal’s response to the SICTT should improve our understanding of the current testing program in Ireland”

Factors associated with the risk of, and animal-level response to, *M. bovis* in Irish cattle

Principal investigators: James O’Keeffe, Paul White (CVERA) and Wayne Martin (University of Guelph)

Although the control of tuberculosis is mainly a herd-level issue, the detection of disease is heavily reliant on testing individual cattle with the single intradermal comparative tuberculin test (SICTT). The purpose of this work is to identify those factors that have an important influence on the animal’s response to the SICTT. The major outcomes in our analyses include the risk of an animal becoming a standard reactor, the skin change (response) to the SICTT, the percentage of SICTT standard reactors among all reactors, and the tuberculous lesion risk at slaughter. The risk factors to be considered include year, month, county or area (represented by the District Veterinary Office (DVO)), class of animal (cow, heifer, steer, bull), breed type (Friesian versus other), factory (slaughterhouse), and reason for the test (test type).

Bovine tuberculosis in alpaca

Principal investigators: Dónal Connolly (Gort Veterinary Clinic), Eoin Ryan (UCD), Simon More (CVERA), Ascinta Kilroy and Martin Hayes (DAF)

“An outbreak of TB in alpaca is currently being Investigated”

Alpaca farming is an emerging animal-based industry on the island of Ireland. Alpaca are a domesticated form of the vicuña, a South American camelid, and produce fibre of high quality and quantity. Based on anecdotal information, alpaca have been considered resistant to infection with *Mycobacterium bovis*. Tuberculosis was recently diagnosed in an Irish alpaca, prompting a detailed investigation of the outbreak.

An understanding of the spatial distribution of TB prevalence in badgers

Principal investigators: Guy McGrath (CVERA), Denise Murphy and Leigh Corner (UCD)

The control of the transmission of *Mycobacterium bovis* from badgers to cattle is currently being achieved through focused culling, following a detailed veterinary epidemiological investigation. As part of the national programme, work is currently underway towards the use of BCG vaccination to control TB infection in badgers. This process would be greatly aided if the geographic distribution of infection in the badger population were understood. This study specifically seeks to determine whether there is spatial variation in the prevalence of tuberculosis in badgers.

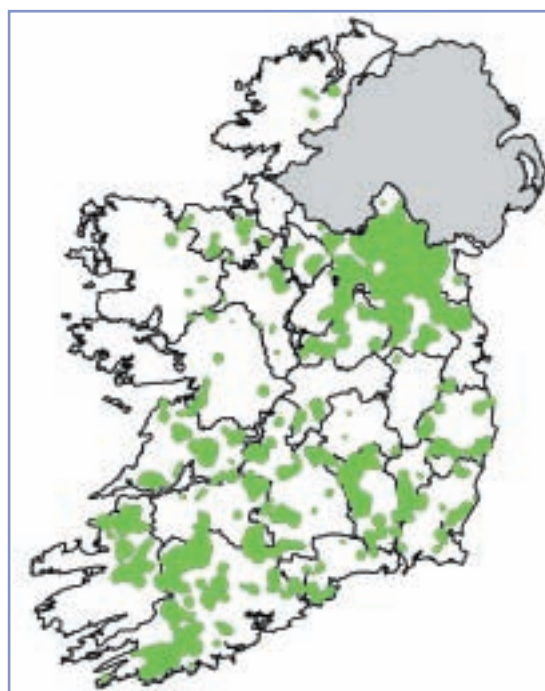
“We require an understanding of the spatial variation in TB prevalence in badgers as we plan for the use of BCG vaccine in badgers”

Work of the Wildlife Unit

Principal investigator: James O'Keeffe (CVERA)

A compulsory national bovine tuberculosis eradication program has been operating in the Republic of Ireland since 1959. Substantial progress was achieved in the early decades, but since the mid 1970s there has been no improvement despite the continuing application of a very intensive national tuberculin testing program. Geographical information systems techniques including kernelling have been used to identify areas of the county where tuberculosis is consistently identified at high incidence levels. Each year, circa 70% of all standard "skin test" reactors are drawn from roughly 30% of the area of agricultural land. Local densities of badgers are being reduced, with removal operations being more intense in areas of the country where TB has been particularly problematic. In the short term, this will result in lowering the risk of cattle herds becoming infected with TB from TB infected badgers in the local environment. The frequency of significant cattle/badger interactions will be reduced as a consequence of the local reduction in the density of both cattle and badgers. Badger interventions are carefully planned and rigorously monitored and only take place as a sequel to an epidemiological investigation carried out by State Veterinarians who must follow a standardised protocol. The medium term strategy targets a 25-30% reduction in the national badger population. This strategy will be re-visited when the results of planned vaccine trials have been evaluated.

“Reactive badger removal forms part of the short term strategy to control bovine tuberculosis in Ireland”



Areas of Ireland where TB in cattle is consistently identified at a high incidence

New work

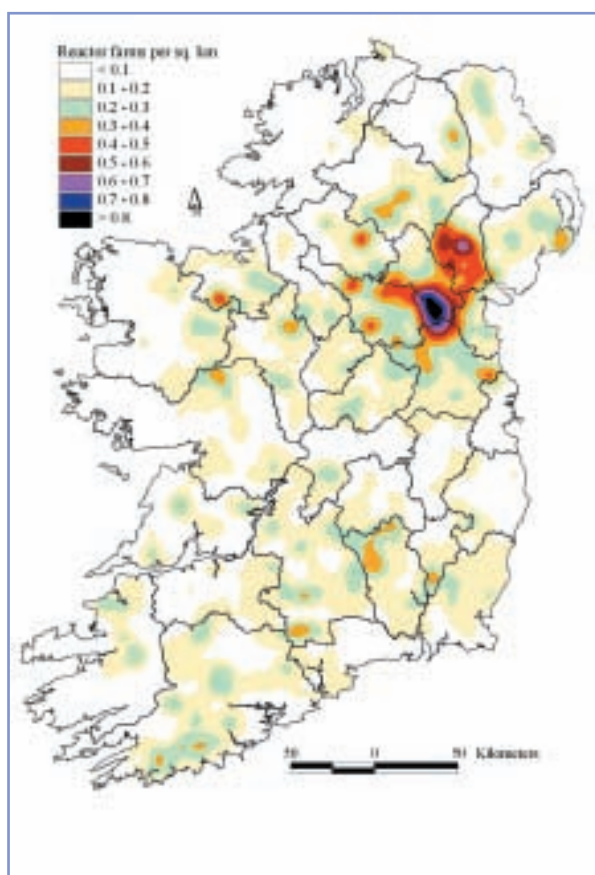
New measures of progress

Principal investigators: Simon More and Isabella Higgins (CVERA)

In recent years, there have been substantial advances in the analysis and management of the national tuberculosis database in Ireland. There has also been a progressive improvement in methods at the Centre for Veterinary Epidemiology and Risk Analysis to manage and analyse this complex database, including the development of new episode-based perspectives in data analysis. Recent meetings have recommended a shift towards herd-based measures, and a separation of surveillance- and control-related activities. Based on these recommendations, this study will investigate alternative (and/or additional) measures of progress, to provide decision-makers with timely and objective information relevant to programme management.

North-South collaborative mapping

Principal investigators: Guy McGrath (CVERA), Darrell Abernethy (DARDNI) and Simon More (CVERA)



Density of reactor farms per square km during 2000
(kernel density with search radius at 10km)

The objective of this study is to spatially represent the island wide distribution of bovine tuberculosis and brucellosis. Both the Republic of Ireland (Department of Agriculture and Food) and Northern Ireland (Department of Agriculture and Rural Development, Northern Ireland) produce map-based reports portraying spatial and temporal representations of tuberculosis and brucellosis within their respective jurisdictions. The structure of these maps vary from simple choropleth maps representing values at a defined administrative level (parish, electoral division, county) to more complex maps derived from spatial analyses of disease point data. With collaborative effort, disease classifications and selection criteria could be made uniform to enable a whole-of-Ireland approach to map-based reporting. Much of the border between Northern Ireland and the Republic of Ireland straddles agricultural land. Identifying disease clusters or trends in disease movement is impossible in areas close to the border. Consolidating and standardising these data from North and South will make it possible to visually compare the true differences and/or similarities in disease levels over time.

Reactive badger removal and levels of TB in cattle herds in Co. Laois

Principal investigators: Francisco Olea-Popelka, Wayne Martin (University of Guelph) and James O'Keeffe (CVERA)

Reactive badger removal forms part of the short-term strategy to control bovine tuberculosis in Ireland. This study will investigate the impact of reactive badger removal on subsequent bovine tuberculosis in cattle herds in Co. Laois.

Tuberculin registration

Principal investigators: Margaret Good (DAF) and Isabella Higgins (CVERA)

The Irish Medical Board requires documentation to support the use of tuberculin as part of the national eradication programme. Field testing data are currently being analysed to demonstrate that the Single Intradermal Comparative Tuberculin Test is a safe test suitable for use in cattle of all sexes, breeds and ages and at all stages of pregnancy and/or lactation. The data analysis also provides support for the contention that tuberculin testing does not affect the fertility or fecundity of bovine animals. It is also necessary to state that there were no adverse incidents reported to the Department of Agriculture in any county in 2003 as a consequence of the injection of tuberculin.

Captive badger studies to compare the Danish and Pasteur strains of BCG

Principal investigators: Denise Murphy, Leigh Corner, Eamonn Gormley (UCD), Eamonn Costello (DAF)

All studies to date in badgers have used the Pasteur strain of BCG but the Danish strain is the only one commercially available in Europe. A vaccination and challenge study will be conducted to compare the performance of the two strains.

Further studies to assess injuries to badgers due to capture in stopped restraints

Principal investigators: Denise Murphy, Leigh Corner (UCD), James O'Keeffe (CVERA)

Animal welfare is an important aspect of the badger culling programme. Results from a previous study reported elsewhere in this publication indicate that the incidence and severity of injuries due to capture in restraints is low. Additional risk factors will be investigated to determine their significance on the level of injuries seen.

Study of the source of infection in cattle breakdowns

Principal investigators: Tracy Clegg and Simon More (CVERA)

Although badgers are an important source of tuberculosis to Irish cattle, other sources of infection are also important. Using data from the four area project, this study will seek to determine the probable cause of outbreaks in the removal areas of the four area project (from which badgers had been removed over a five year period), and to investigate possible temporal associations between badger removal and outbreak source in these areas.

Investigating an outbreak of TB with human involvement

Principal investigators: Paul Doran (DAF) and Simon More (CVERA)

In Ireland, bovine tuberculosis rarely infects people. Nonetheless, because there is still a risk of infection from cattle to people, it is important that any such cases are investigated in detail. This work concerns one such case, to determine the method of spread and of lessons to be learned to minimise the likelihood of similar cases into the future.

Badger distribution and abundance: results from the four area project

Principal investigators: Paddy Sleeman (University College Cork), Simon More, Tracy Clegg and Dan Collins (CVERA)

Preliminary results from the four area project suggest that the abundance of badgers is substantially less than that predicted in earlier national surveys. Therefore, this study will seek to establish the distribution and abundance of badgers during the first two years of the four area project. Distribution will be established based on sett location; further, in the removal areas badger distribution will be based on actual badger capture, and in the reference area on detected badger activity. Abundance estimated by captures will only be possible in the removal areas. Preliminary results indicate that setts were smaller than those found in Britain; further, they are very frequent in hedges and most are accessible to cattle. Badgers in Donegal were more common than expected.

The impact of badger removal in the four area project

Principal investigators: Paddy Sleeman (University College Cork) and Simon More (CVERA)

The aim of this study is to describe the impact of badger removal on both the distribution and abundance of remaining badgers. The study will focus on data collected during the final three years of the four area project. During this period, badgers continued to be captured in all removal areas, with the exception of the north of the Donegal area which was surrounded by sea on three sides. This information will provide a measure of the effectiveness of the removal operations, and of barriers to badger migration such as rivers and mountains.

Winter yard survey

Principal investigator: Paddy Sleeman (University College Cork)

It is suspected that the winter housing of cattle may play a role in the transmission of infection from badgers to cattle. This study is being conducted to survey evidence of badger entry to cattle sheds, based on farmer interview and examination of the farm environment looking for tracks and other signs. To date, there have been few signs of badgers, however, signs (and sometimes cadavers) of wild foxes and rats have been found.

Genetics of predisposition to tuberculosis in Irish dairy and beef cattle

Principal investigators: Donagh Berry (Teagasc), Margaret Good (DAF), Andrew Cromie (ICBF) and Simon More (CVERA)

There are substantial gaps in knowledge concerning the genetics of tuberculosis susceptibility in cattle, and no study has yet evaluated the mode by which susceptibility to bovine tuberculosis is inherited, nor the regulation of expression of bovine polygenes across differing environments. There are several large animal- and herd-level datasets (animal breeding, disease control) in Ireland which represent an opportunity, unique internationally, to address some of these gaps in knowledge. Using these datasets, we aim to quantify the heritability (both direct and maternal) for susceptibility to tuberculosis.

Quantification of bovine tuberculosis transmission in Irish cattle herds and optimising the eradication program (Chapters 2 – 5 of a PhD.)

Principal investigators: Paul White (CVERA), Klaas Frankena (Wageningen University)

The first chapter of the PhD has been considered previously (Descriptive statistics, 1995-2004). The objectives of the subsequent chapters include:

- to quantify the contribution of introduced, possibly infected animals as source of herd breakdown (Chapter 2);
- to incorporate the concept of the reproduction ratio (R_0) into measurement of the between-herd and within-herd spread of tuberculosis (Chapter 3);
- to estimate the component badgers contribute to the observed levels of tuberculosis evident in cattle herds (Chapter 4); and
- based on the results of previous chapters, to adopt a modelling approach to combine the risk factors from various sources, and calculate an overall R_0 value (Chapter 5)

National badger census

Principal investigator: Guy McGrath (CVERA)

The objective of this study is to estimate the current population of badgers in the Republic of Ireland. An up-to-date census of population data are required to assess the impact of badger removal activities conducted by the Department of Agriculture through the bovine tuberculosis eradication scheme. Geographical Information System modelling techniques will be used to extrapolate a total population from known populations within surveyed sites.

The diet of badgers (*Meles meles*) in Ireland

Principal investigator: Grainne Cleary, Nicola Marples (TCD), James O'Keeffe (CVERA) and Leigh Corner (UCD)

The objective of this study is to describe the diet of badgers in Ireland over the 12 month period commencing March 2005 until February 2006. Badgers were sampled from selected counties in the north and south of Ireland, and the study will compare diets (both qualitatively and quantitatively) within and between these regions. Dietary components will be estimated from samples taken from the stomachs and large intestines of badgers, and estimates of the correlation between the findings at both sites will be established.

Factors associated with reproduction in badgers (*Meles meles*) in Ireland

Principal investigator: Lynsey Stuart, Nicola Marples (TCD) James O'Keeffe (CVERA) and Leigh Corner (UCD)

The objective of this study is to describe the reproductive cycle and reproductive efficiency of a sample of Irish badgers taking into account such factors as geographic area, age, body condition, group size, diet and disease status. The badgers in this study are drawn from the sample used for the dietary study.

The effect of bovine TB on milk production

Principal investigators: Fiona Boland, Gabrielle Kelly (UCD), Donagh Berry (Teagasc), Andrew Cromie (ICBF) and Simon More (CVERA)

The main aim of this study is to assess the effect of infection with bovine TB on milk production in dairy cows. The period of study is 1st November 2000 to 31st October 2005. Herds testing positive in the period with at least two positive animals will be identified from the CVERA database and matched to the ICBF database. From this database, information on milk production and other animal and herd level traits will be extracted for all animals in the infected herds. The resulting dataset will have a defined hierarchy: herd-level, animal-level, lactation-level. The statistical analysis will focus on (a) comparing infected animals with non-infected adjusting for herd-level variables i.e. cross-sectional comparisons and (b) longitudinal comparisons i.e. assessing changes over time within animals. Statistical methods will include the use of generalised linear models with random effects (glmm).

Spatial analysis of bovine TB data – the effect of badger removal

Principal investigators: Syed Zeeshan Haider Zaidi and Gabrielle Kelly (UCD)

The aim of this study is to assess if TB clusters in herds and badgers and if any associations between these occur. We examine data from the Four Area Project. The analysis will identify a suitable distance measure between herds with the aid of the GIS. Comparisons will be made between restricted and non-restricted herds, removal and reference areas and over counties. Clustering in both time and space will be examined. Similarly the spatial distribution of badgers (infected/ non-infected setts) and association with herd breakdowns will be examined. This will build on the work of Olea-Polpeka et al. (2003, 2005) using more sophisticated statistical methods. The analysis will involve programming in SAS and R as the data does not fall into a format suitable for applying existing software (such as SatScan). Finally, statistical models derived in the FAP concerning the risk of restriction for a herd will be extended to include some measure of contiguity to setts/infected setts and to neighbouring restricted herds. The results of the work will be compared to the results from the RBCT in the U.K.

The Greenfield study

Principal investigators: Denise Murphy (UCD), Guy McGrath (CVERA), Leigh Corner and Eamonn Gormley (UCD)

The study will test the hypothesis that infection prevalence in cattle can be used to identify infection prevalence in badger populations. To date, areas of high prevalence of infection in cattle have been examined through badger culling associated with herd breakdowns. This project will focus on the infection prevalence in badgers in areas of low prevalence of infection in cattle.

Key meetings/presentations

Tracy Clegg

- Tuberculosis review workshop, Athlone (26-27 April 2004)
- Annual Conference, Society for Veterinary Epidemiology and Preventive Medicine, Nairn (30 March – 1 April 2005)
- 4th International Conference on *Mycobacterium bovis*, Dublin (22-26 August 2005)
- DAF meeting (pre-movement study), Dublin (10 October 2005)
- Irish Farmers Association (Wildlife Unit), Portlaoise (25 November 2005)

Leigh Corner

- DG Sanco review visit, Kilkenny (9 June 2004)
- National Parks and Wildlife Division, Department of Environment, Heritage and Local Government, Tallaght, (14 April, 2005)
- 4th International Conference on *Mycobacterium bovis*, Dublin (22-26 August 2005)
- DAF wildlife meeting, Kilkenny, Cork (27-28 September 2005)
- DARDNI meeting, Belfast (1 July 2004)
- Role of Badgers in the Epidemiology of Tuberculosis in Domestic Livestock, EpiCentre, Massey University, New Zealand (9 December, 2005)
- DAF meeting (ER76 workshop), Carlow (24 September 2004)
- Defra TB Forum, London (23 February 2005)
- Irish Farmers Association (Wildlife Unit), Portlaoise (25 November 2005)

Eamonn Gormley

- DG Sanco review visit, Kilkenny (9 June 2004)
- Visit to Texas A & M University (23 Oct – 7 Nov 2004)
- World TB symposium, Mater Hospital (24 March 2005)
- 4th International Conference on *Mycobacterium bovis*, Dublin (22-26 August 2005)
- DAF wildlife meeting, Kilkenny, Castleblaney (27-29 September 2005)
- DEFRA TB Vaccine Advisory Group meeting (8 November 2005)
- Irish Farmers Association (Wildlife Unit), Portlaoise (25 November 2005)

Gabrielle Kelly

- Conference of Applied Statistics in Ireland, Enniskillen (18-20 May, 2005)

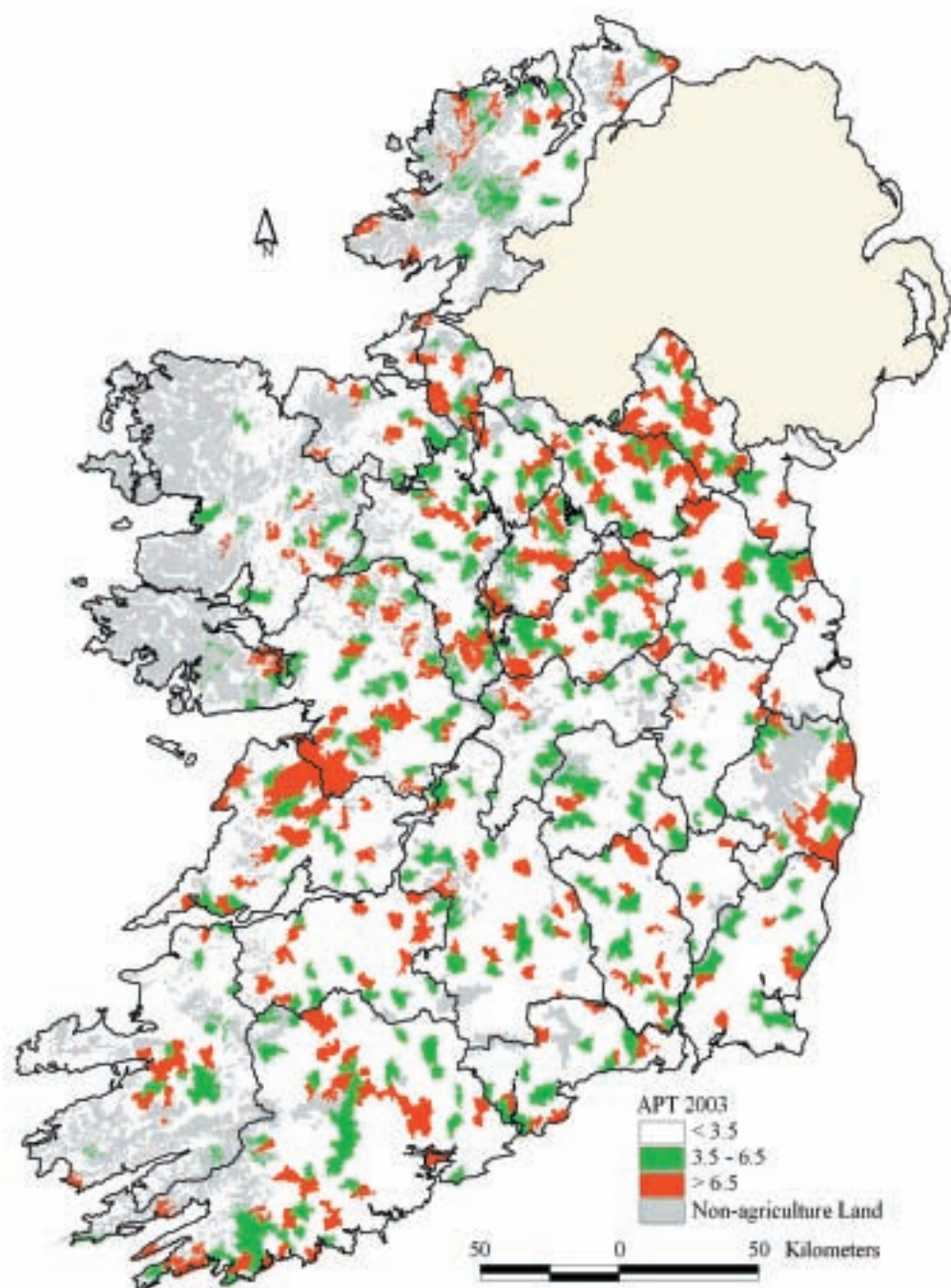
Simon More

- Regional DAF meetings (Kilkenny, 6 February 2004; Cavan, 11 February 2004; Carrick-on-Shannon, 12 February 2004; Thurles, 20 May 2004)
- Tuberculosis review workshop, Athlone (26-27 April 2004)
- DG Sanco review visit, Kilkenny (9 June 2004)
- DAF meeting (ER76 workshop), Carlow (24 September 2004)
- DAF meeting (following DG-Sanco review visit), Clare (9 October 2004)
- Defra visit, Dublin (13 October 2004)
- 35th UNION World Conference on Lung Health, Paris (28 October -1 November 2004)
- Defra TB Forum, London (23 February 2005)
- DAF meeting (results of the four area project), Dublin (16 March 2005)
- Veterinary Laboratories Agency, Weybridge (18 March 2005)
- Annual Conference, Society for Veterinary Epidemiology and Preventive Medicine, Nairn (30 March – 1 April 2005)
- Warwick University visit, Warwick (5-6 July 2005)
- 28th World Veterinary Congress, Minneapolis (14-20 July 2005)
- 4th International Conference on *Mycobacterium bovis*, Dublin (22-26 August 2005)
- DAF meeting (pre-movement study), Dublin (10 October 2005)
- Veterinary Ireland meeting (results of the four area project), Letterkenny (13 October 2005)
- Irish Farmers Association (Wildlife Unit), Portlaoise (25 November 2005)

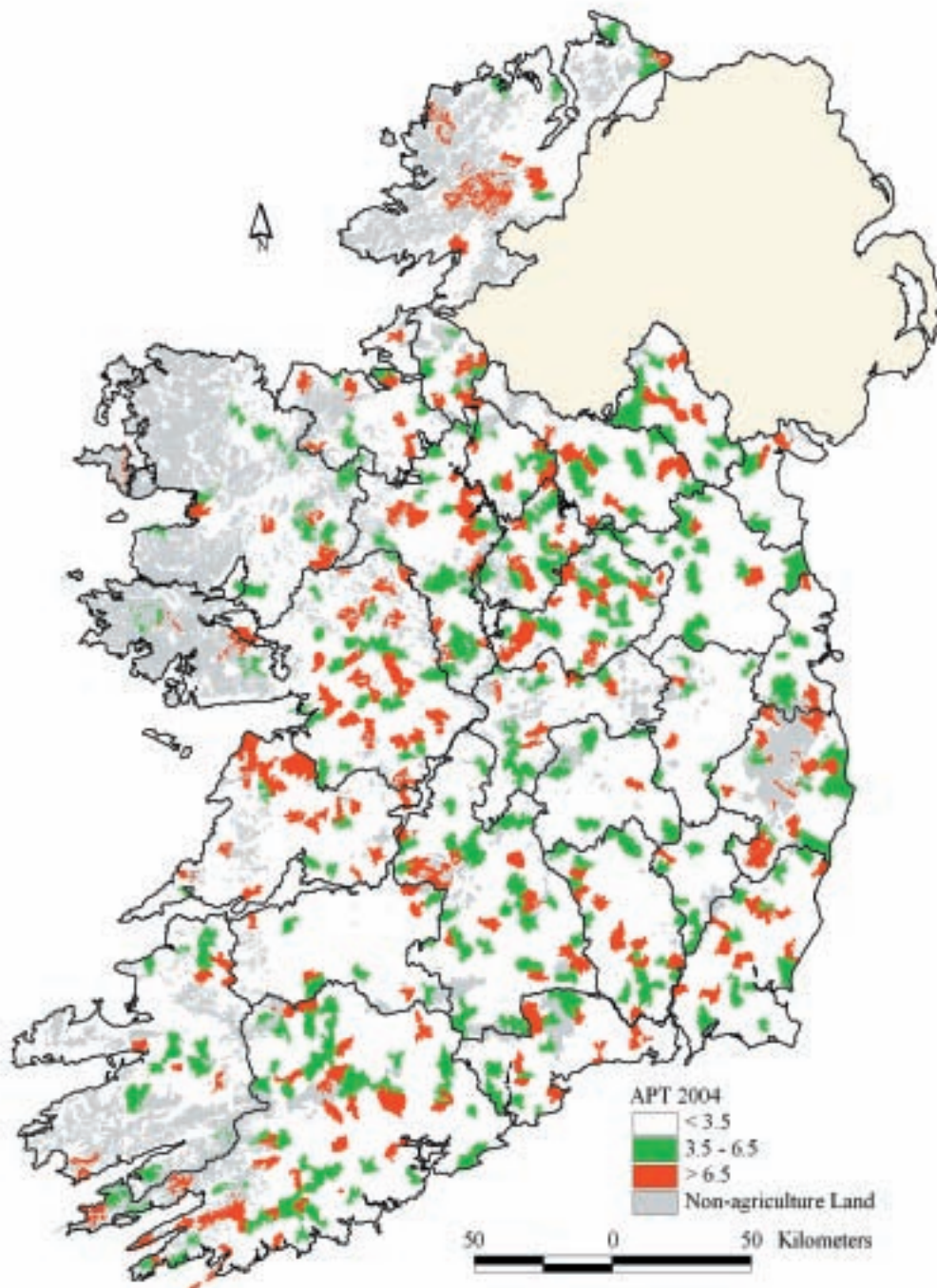
Paul White

- Warwick University visit, Warwick (5-6 July 2005)
- Annual Conference, Society for Veterinary Epidemiology and Preventive Medicine, Nairn (30 March-1 April 2005)

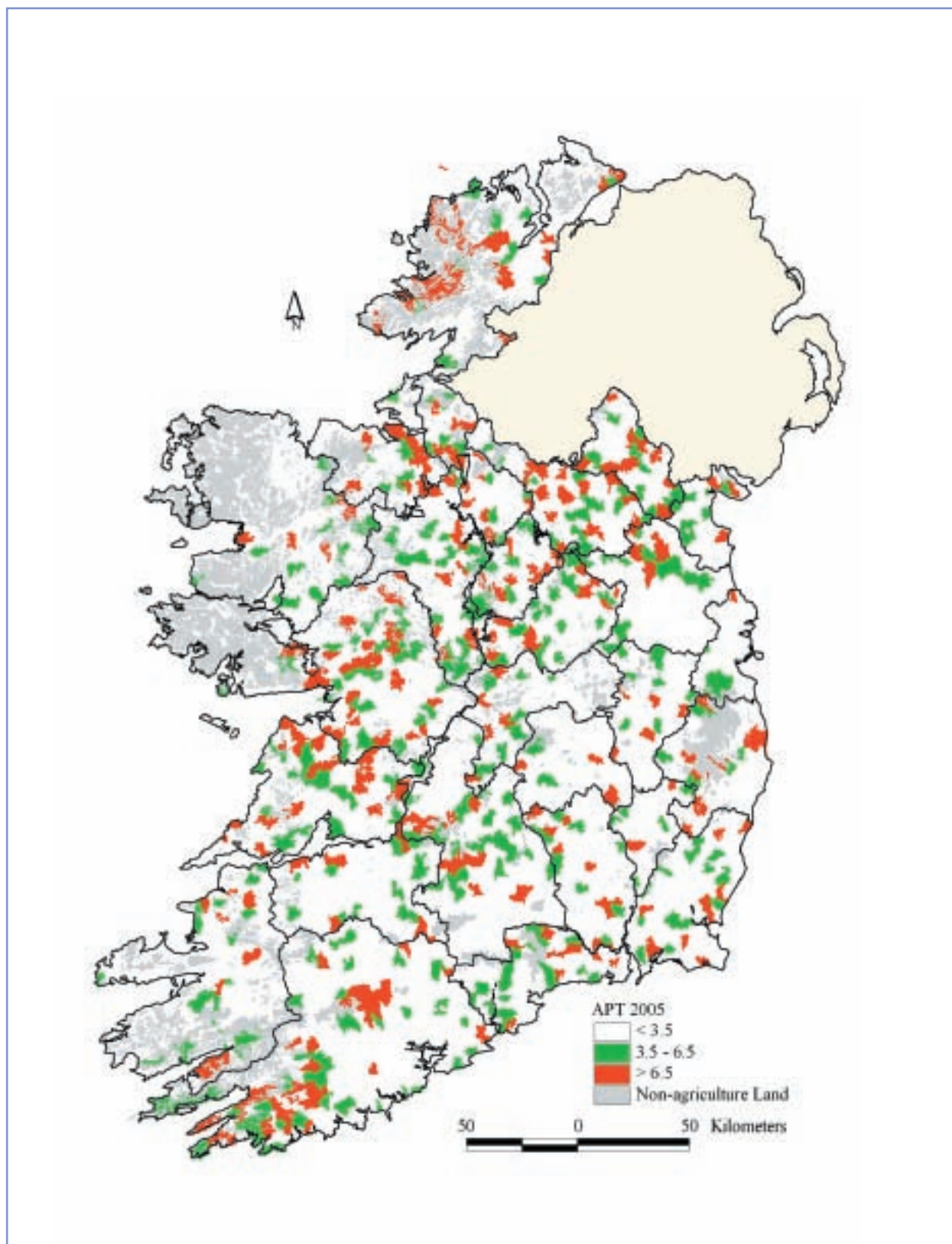
National maps



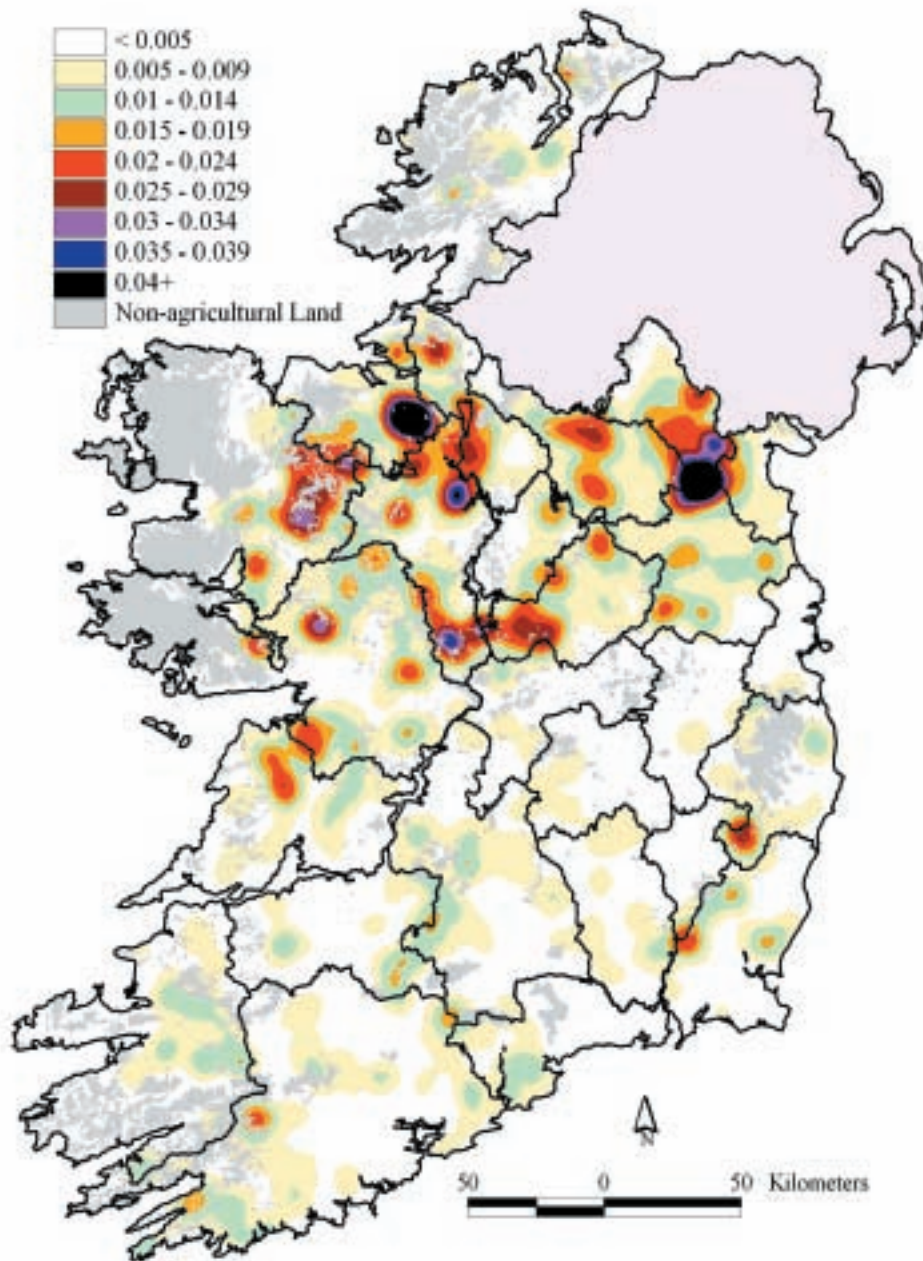
APT (reactors per 1000 tests) per district electoral division, 2003



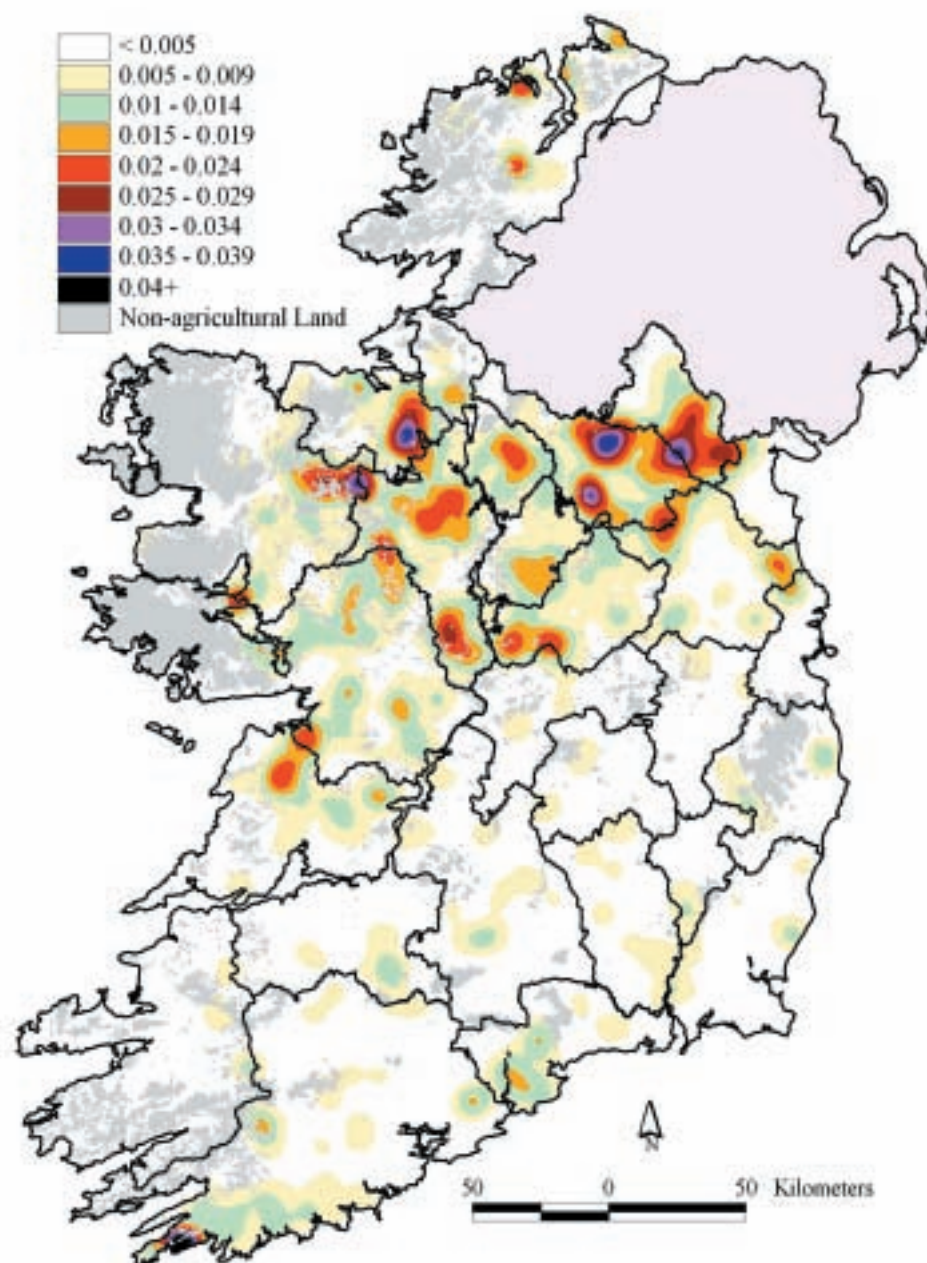
APT (reactors per 1000 tests) per district electoral division, 2004



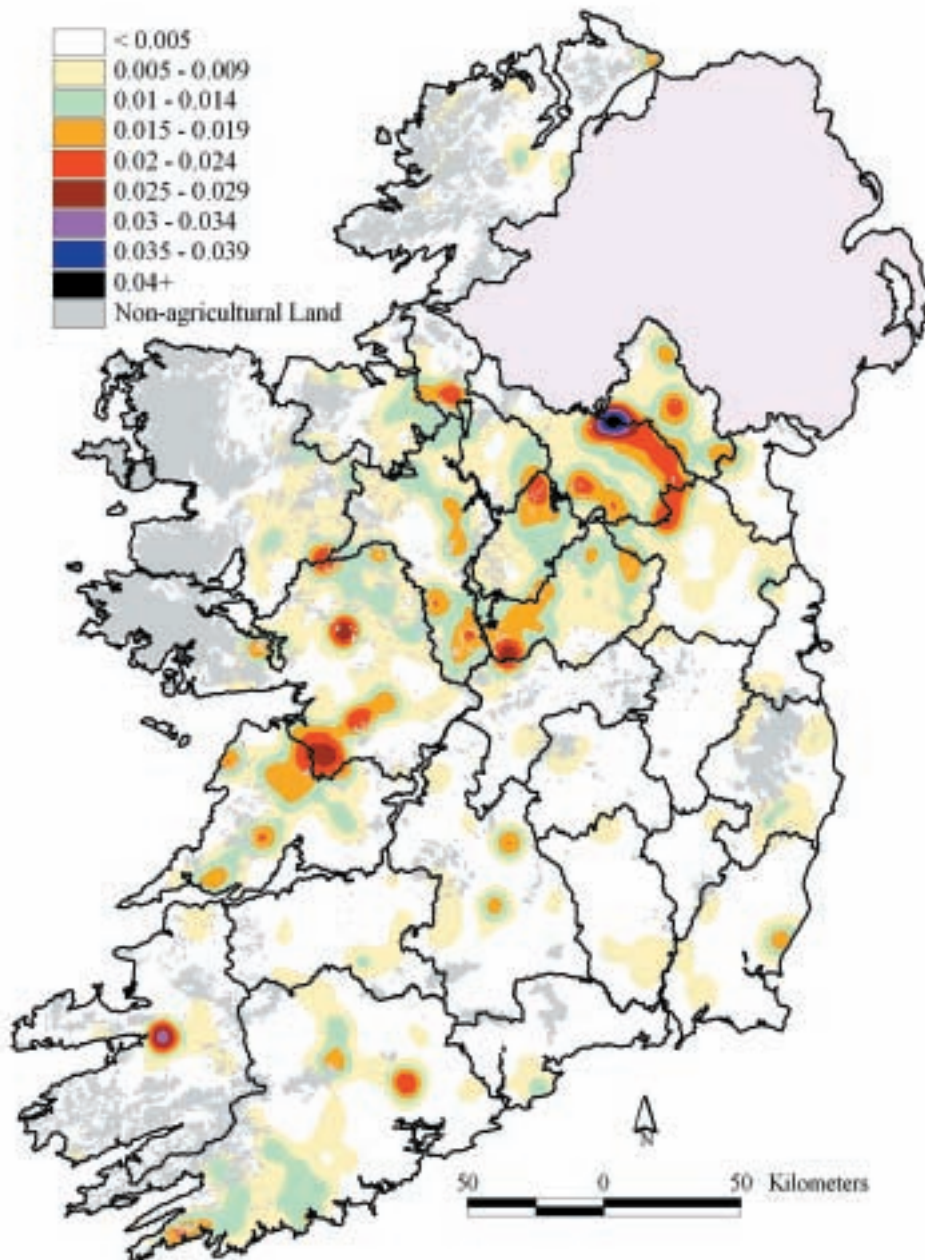
APT (reactors per 1000 tests) per district electoral division, 2005



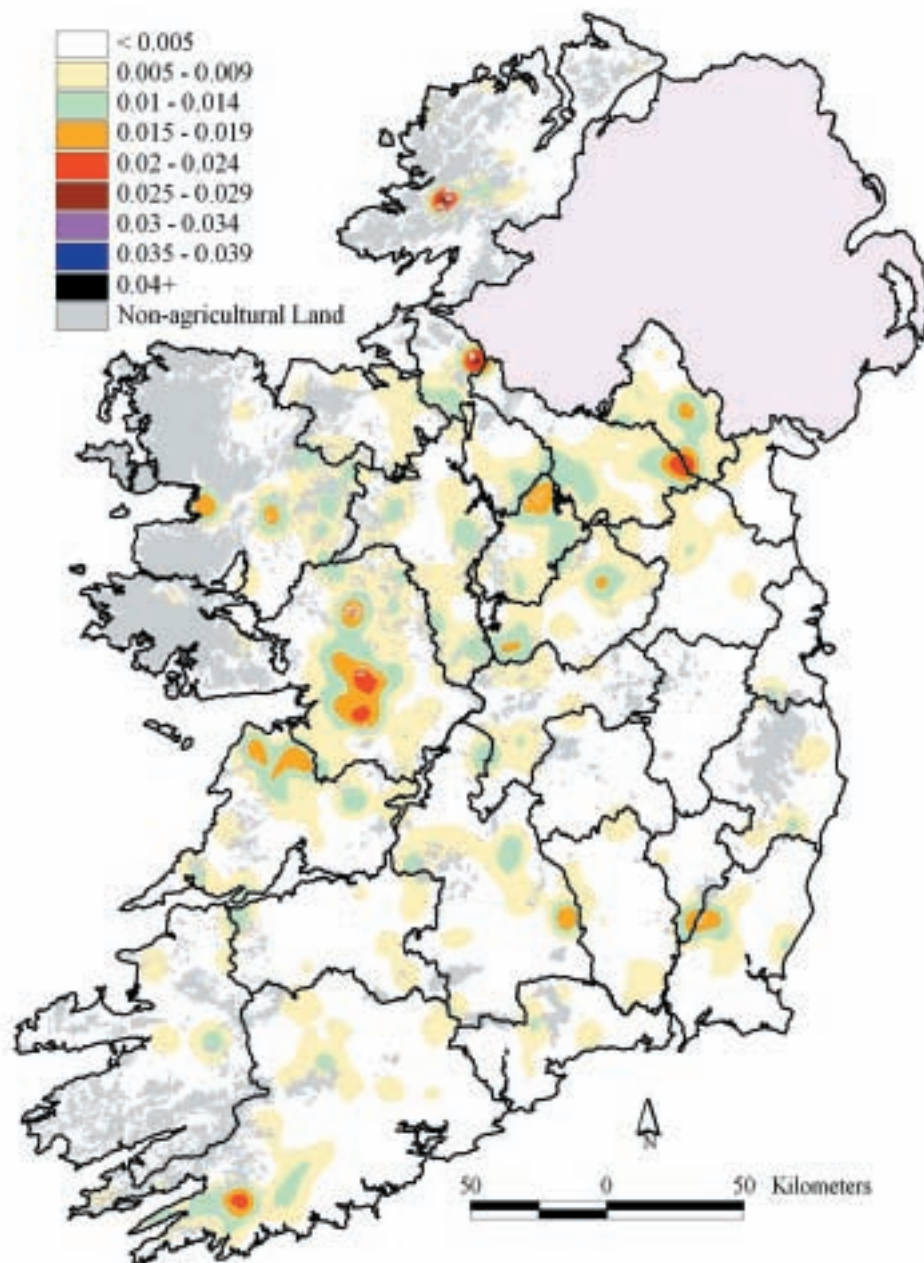
Density of TB incidence per square km during 2001 (kernel density with search radius at 10km)



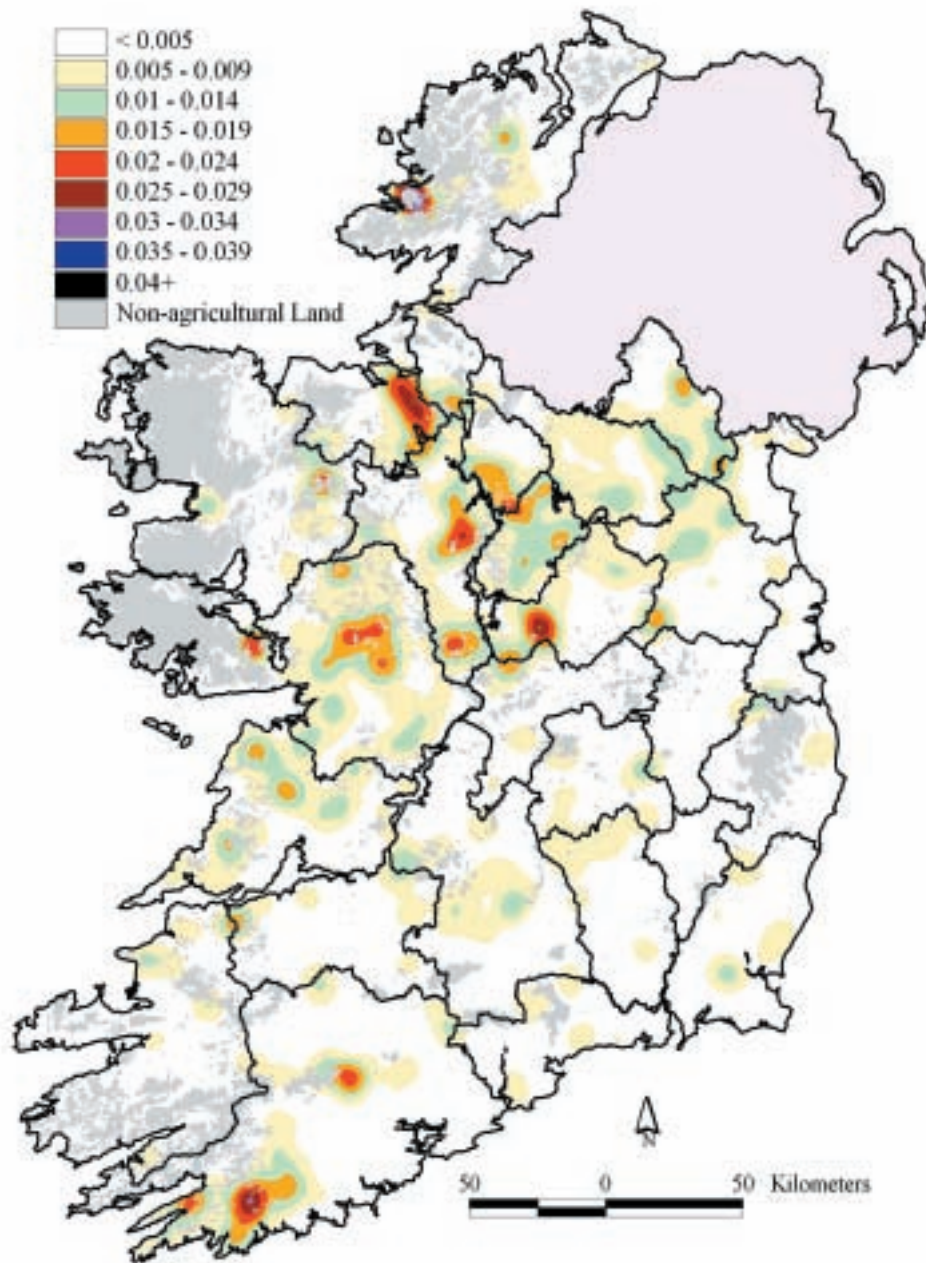
Density of TB incidence per square km during 2002 (kernel density with search radius at 10km)



Density of TB incidence per square km during 2003 (kernel density with search radius at 10km)



Density of TB incidence per square km during 2004 (kernel density with search radius at 10km)



Density of TB incidence per square km during 2005 (kernel density with search radius at 10km)