



BOVINE TUBERCULOSIS

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BADGER ECOLOGY

HOW MANY EURASIAN BADGERS (*MELES MELES*) ARE THERE IN IRELAND?

a. An ecological approach

Paddy Sleeman, John Davenport (UCC Zoology), Simon More, Tracy Clegg, Daniel Collins (UCD CVERA), John Griffin, Ian O'Boyle (DAFF)

An understanding of the total number of badgers in Ireland is important, noting the contribution of this species to the epidemiology of bovine tuberculosis. Using data from the four area project, this study seeks to ascertain the size of Irish badger social groups, and thereby more accurately estimate the size of the badger population of Ireland.

b. A GIS approach

Guy McGrath (UCD 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 DAFF 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 REPRODUCTIVE CYCLE OF THE MALE AND FEMALE EURASIAN BADGER (*MELES MELES*) IN IRELAND

Lynsey Stuart, Nicola Marples (TCD Zoology), Leigh Corner (UCD Agriculture, Food Science and Veterinary Medicine), James O'Keeffe (DAFF)

The aims of this project are to describe the annual reproductive cycle of the male and female Eurasian badger, *Meles meles*, in Ireland, to determine the reproductive potential of the badger population and to develop an understanding of the factors that may affect its reproductive success. The results to date suggest the Irish badger population has a high reproductive potential, which is neither affected by reproductive competition between individuals nor by infection with tuberculosis. The high reproductive potential is likely to be related to the low density of the Irish badger population.

SEASONALITY OF PARTURITION IN THE EURASIAN BADGER (*MELES MELES*)

Rosario Carroll, Nicola Marples (TCD Zoology), Leigh Corner (UCD Agriculture, Food Science and Veterinary Medicine), James O'Keeffe (DAFF)

The primary aim of this project is to predict the annual cubbing times in Irish badger populations and to investigate if the cubbing period varies from year to year. This will enable DAFF to limit the culling of lactating females during the months when the dependent cubs are most vulnerable. The study will also investigate the differences in badger group composition between undisturbed groups and newly established groups. This will provide information concerning the diversity in foraging activity and social interactions in both groups.

BAIT-MARKING AND LIVE TRAPPING STUDIES DURING THE FOUR AREA PROJECT

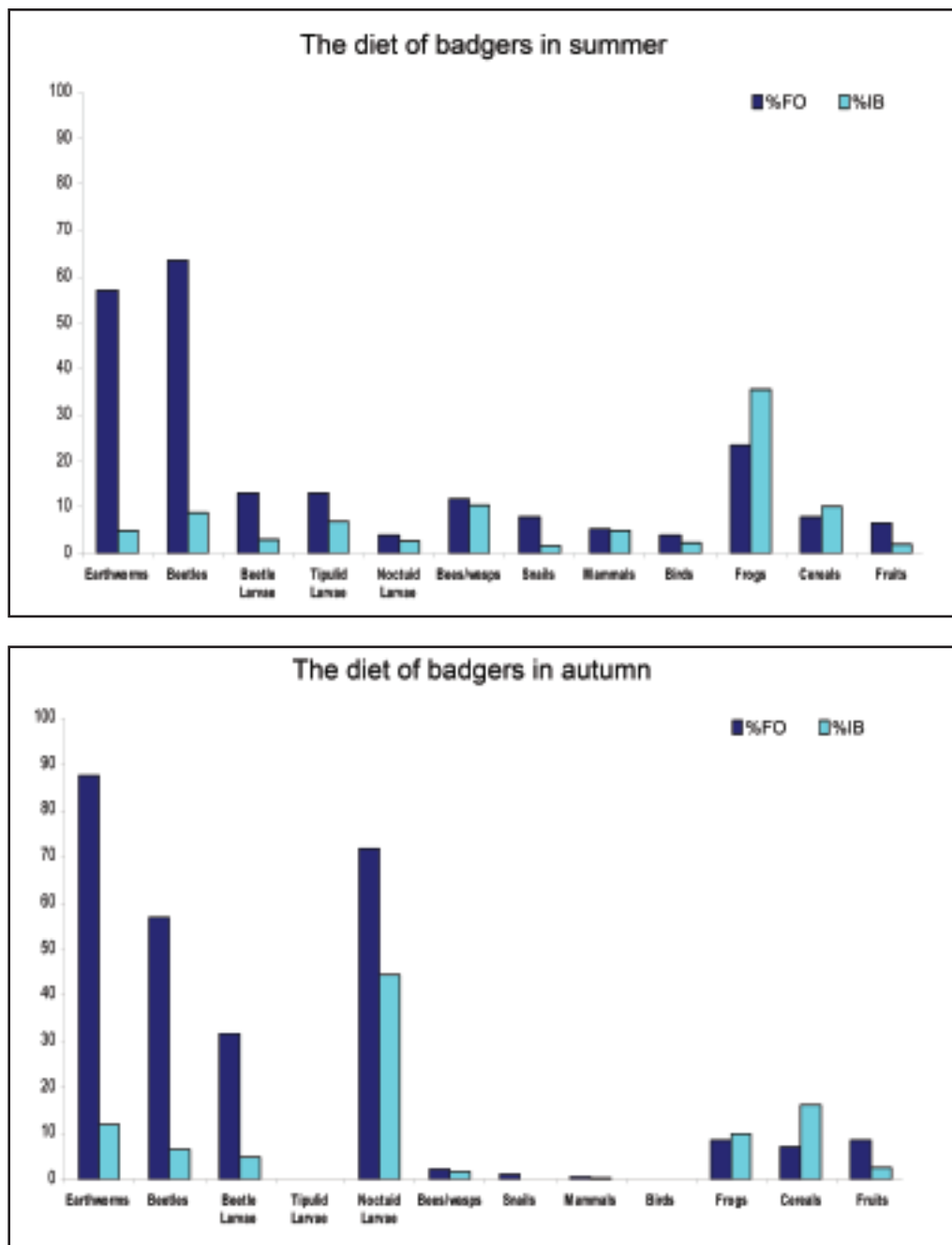
Chris Smal, Julian Brown (independent consultants), John Griffin, Ian O'Boyle (DAFF), Paddy Sleeman (UCC Zoology)

During the four area project, badger social groups were identified using bait-marking and live trapping. These results are now being documented.

THE FEEDING STRATEGIES AND FORAGING BEHAVIOUR OF THE EURASIAN BADGER (*MELES MELES*) IN IRELAND

Gráinne Cleary, Nicola Marples (TCD Zoology), Leigh Corner (UCD Agriculture, Food Science and Veterinary Medicine), James O'Keeffe (DAFF)

Although the Eurasian badger (*Meles meles*) has been studied in many of its range of habitats across Europe, a detailed examination of its feeding strategies and foraging behaviour has not been conducted in Ireland. The results of this comprehensive investigation into the diet of badgers demonstrate that they opportunistically consume many prey items throughout the year. However, during spring and autumn, badgers display strong preferences for seasonal insect larvae, in particular Tipulids and Noctuids. They are probably best described as generalist foragers displaying seasonal preferences.



Seasonal variation in the diet of badgers

(%FO = number of stomachs found to contain a given item expressed as a percentage of the total number of stomachs, %IB = proportions of food categories in the stomach sample in relation to the total stomach contents)

BADGER-TO-BADGER TRANSMISSION

GENOTYPING OF *MYCOBACTERIUM BOVIS* ISOLATES FROM BADGERS IN FOUR AREAS OF THE REPUBLIC OF IRELAND BY RESTRICTION FRAGMENT LENGTH POLYMORPHISM ANALYSIS

Costello, E., Flynn, O., Quigley, F., O'Grady, D., Griffin, J., Clegg, T., McGrath, G.

Veterinary Record 159 (2006), 619-23.

An analysis of the molecular epidemiology of *Mycobacterium bovis* isolated from badgers was made in four selected areas of the Republic of Ireland in which an intensive badger removal programme was being carried out over a period of five years. Tissue samples from 2310 badgers were cultured. Restriction fragment length polymorphism (RFLP) analysis using IS6110, polymorphic GC-rich sequence (PGRS) and direct repeat sequence (DR) probes was applied to the isolates from 398 badgers, and 52 different RFLP types were identified. Most of the isolates belonged to seven predominant types. The other 45 types were represented by few isolates. An analysis suggests that some of these 45 types may have been introduced by the inward migration of badgers and others may have been the result of genetic changes to one of the prevalent types. The badgers were divided into groups on the basis of the sett at which they were captured, and RFLP typing was applied to isolates from two or more badgers from 85 groups. Multiple RFLP types were identified among isolates from 50 of these groups, suggesting that badgers probably moved frequently between group territories.

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“The results of the RFLP analyses support the hypothesis that there is a considerable level of extraterritorial movement by badgers.”

THE PATHOGENESIS OF *MYCOBACTERIUM BOVIS* INFECTION IN WILD BADGERS

Denise Murphy, Leigh Corner (UCD Agriculture, Food Science and Veterinary Medicine)

The European badger (*Meles meles*) is recognised as the principal wildlife reservoir of *M. bovis* infection in Ireland. Understanding of the pathogenesis of the disease in naturally infected badgers will help to decipher transmission pathways between badgers and cattle, which will be essential for the successful development and application of a vaccine. In the present study, we have investigated the prevalence and distribution of *M. bovis* infection in a group of naturally infected wild badgers (n=215). The findings of this study confirm that most infected badgers have non-visible lesions and the majority of infected badgers with visible lesions are not severely diseased. The distribution of infection indicates that the lungs and/or pulmonary lymph nodes of infected badgers are the principal tissues involved, confirming that tuberculosis in badgers is primarily a respiratory disease.

THE 'GREENFIELD' AND 'REDFIELD' STUDIES

Denise Murphy (UCD Agriculture, Food Science and Veterinary Medicine), Guy McGrath, Daniel Collins (UCD CVERA), Leigh Corner, Eamonn Gormley (UCD Agriculture, Food Science and Veterinary Medicine)

The objective of this study is to test the hypothesis that infection prevalence in cattle can be used to identify infection prevalence in associated badger populations. To date, infection prevalence in badgers has been studied in areas of high prevalence of infection in cattle following badger culling associated with herd breakdowns. The 'Greenfield' study focuses on the infection prevalence in badgers in areas of low prevalence of infection in cattle. The 'Redfield' study concerns the infection prevalence in badgers removed in the first trapping event in areas of high prevalence of infection in cattle. While not directly comparable due to different selection criteria, the results of the studies will identify any significant difference in the prevalence of infection in the two badger populations.

GREENFIELD SITE SELECTION

Guy McGrath (UCD CVERA), Paul White (DAFF), Daniel Collins (UCD CVERA)

The relationship between TB infected badgers and TB breakdowns in cattle have been established in the absence of an understanding of the prevalence of TB in the badger population where there are no significant TB problems in the local cattle population both currently and historically (Greenfield sites). Based on existing assumptions of TB transmission pathways, we would expect to find levels of TB in badgers to be significantly lower in areas where there has never been a TB problem in cattle. If, however, TB levels in badgers from Greenfield areas are found to be the same as those found in close proximity to herd breakdowns attributed to badgers, our understanding of the mode of transmission will need to be re-examined. The aim of this study was to identify areas of land with either no or a very low historical prevalence of TB in local cattle populations (Greenfield sites).

Ireland was divided into 112,440 equal sized hexagons with a maximum cross sectional width of 1 km (Figure 1, highlighting an area in west Co. Cork, east Co. Kerry for illustration). The locations of herds in Ireland are held in polygon format on a spatial database through the Land Parcel Identification System (LPIS). Greenfield study sites were identified using Geographical Information Systems (GIS), following the following exclusions:

- Exclusion of all hexagons associated with herds with a historic TB problem. The TB history of all herds in the country is recorded and maintained within the Animal Health Computer System (AHCS). A unique identifier, the herd number, is used to join the spatial data with the herd TB testing history. A herd was permitted a single TB breakdown of no more than 2 reactors in the 5 years period between 2002 and 2006. Any herds failing to meet this criterion were flagged. A spatial query was then performed identifying any hexagons that touched a flagged herd (Figure 2a)
- Exclusion of hexagons not touching farms stocked with a minimum of 17 animals and maximum of 80 animals (2nd and 3rd quartiles of the population) (Figure 2b)
- Exclusion of all hexagons within 1 km of badger setts treated under the Government's reactive cull strategy (Figure 2c)
- Exclusion of hexagons touching any hexagons excluded in criteria 1 – 3 (Figure 2d)
- Exclusion of hexagons overlaying commonage (common grazing land) (Figure 2e)

The internal hexagon boundaries of the remaining hexagons were then dissolved creating Greenfield areas. Very small 'island' Greenfield areas were excluded. An approximate centroid was calculated for all remaining Greenfield areas. Each centroid was then inspected against a backdrop of geo-rectified orthophotography to ensure it was on or close to agricultural land and assigned a unique identification number (Figure 3).

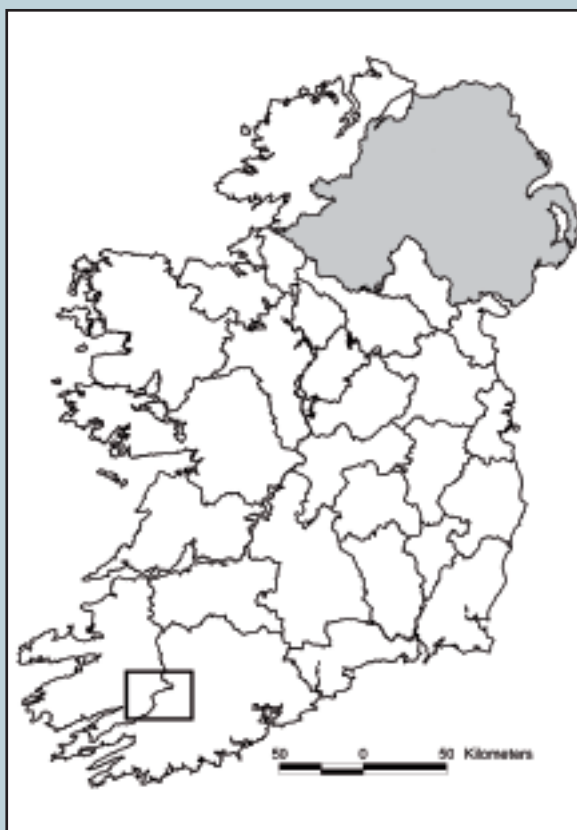


Figure 1. Selected area for illustration

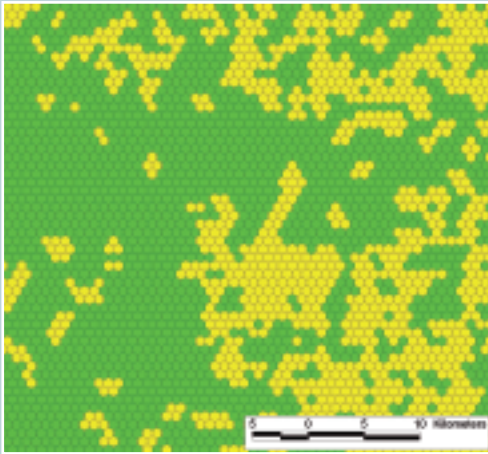


Figure 2a. Greenfield site selection, step 1

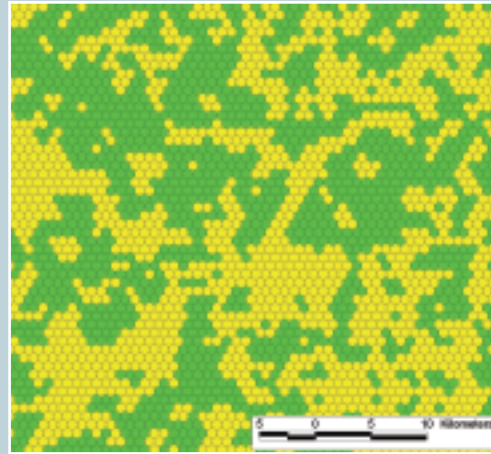


Figure 2b. Greenfield site selection, step 2

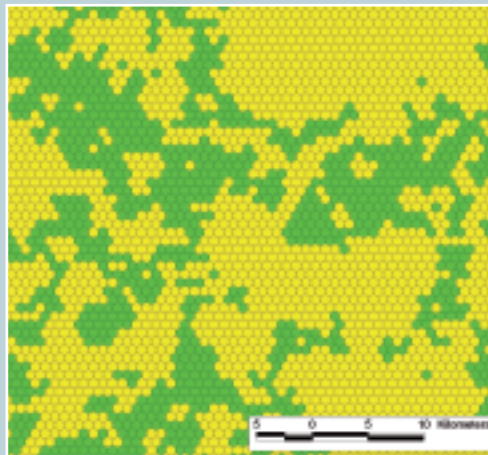


Figure 2c. Greenfield site selection, step 3

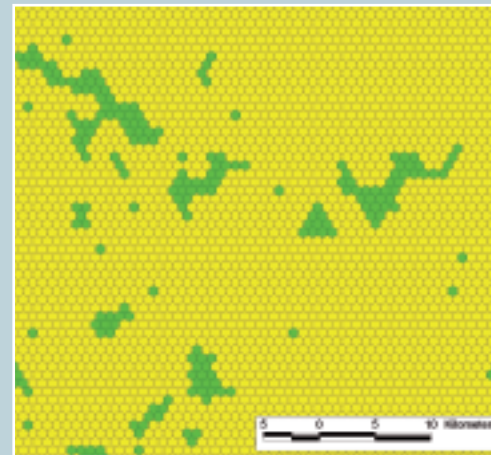


Figure 2d. Greenfield site selection, step 4

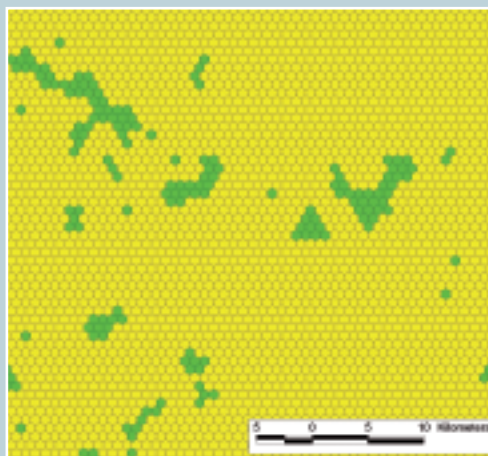


Figure 2e. Greenfield site selection, step 5

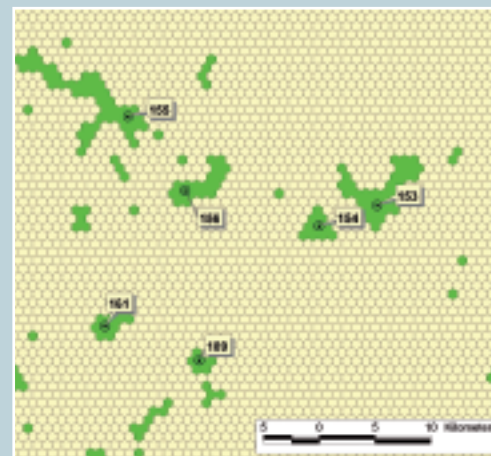


Figure 3. Greenfield areas and identified survey sites

BADGER-TO-CATTLE TRANSMISSION

a. Evidence and understanding

THE ROLE OF WILD ANIMAL POPULATIONS IN THE EPIDEMIOLOGY OF TUBERCULOSIS IN DOMESTIC ANIMALS: HOW TO ASSESS THE RISK

Corner, L.A.

Veterinary Microbiology 112 (2006), 303-312.

Tuberculosis is present in wild animal populations in North America, Europe, Africa and New Zealand. Some wild animal populations are a source of infection for domestic livestock and humans. An understanding of the potential of each wild animal population as a reservoir of infection for domestic animals is reached by determining the nature of the disease in each wild animal species, the routes of infection for domestic species and the risk of domestic animals encountering an infectious dose. The mere presence of infection in a wild animal population does not of itself provide evidence of a significant wildlife reservoir. Although at times counterintuitive, wildlife populations with high disease prevalence may not necessarily have a role in the epidemiology of disease in domestic livestock. The key concepts used in deciding whether an infected wild animal population is involved in the epidemiology of tuberculosis in domestic livestock is illustrated by reference to six well-researched cases: the feral pig (*Suis scrofa*) and feral Asian water buffalo (*Bubalus bubalis*) in Australia, white tailed deer (*Odocoileus virginianus*) in Michigan, and the brushtail possum (*Trichosurus vulpecula*) and other species, such as the ferret (*Mustela furo*), in New Zealand. A detailed analysis of *Mycobacterium bovis* infection in the Eurasian badger (*Meles meles*) in Ireland and its role as a reservoir of infection for cattle is also presented.

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“An understanding of the potential of each wild animal population as a reservoir of infection for domestic animals is reached by determining the nature of the disease in each wild animal species, the routes of infection for domestic species and the risk of domestic animals encountering an infectious dose.”



Mycobacterium bovis has been found in a number of wild animal species, including the kudu

A LONG-TERM OBSERVATIONAL STUDY OF THE IMPACT OF BADGER REMOVAL ON HERD RESTRICTIONS DUE TO BOVINE TUBERCULOSIS IN THE IRISH MIDLANDS DURING 1989–2004

Gabrielle Kelly (UCD Statistics), Joe Condon (DIT), Simon More (UCD CVERA), Leonard Dolan (DAFF), Isabella Higgins (UCD CVERA), John Eves (DAFF)

An observational study is being conducted to critically evaluate the long-term effectiveness of proactive badger culling and to gather insights into the long-term effects of reactive culling, on TB prevalence in cattle. The study is based on data from the Irish midlands since 1989, of badger removal and TB incidence in cattle.

SPATIAL ASSOCIATION OF *MYCOBACTERIUM BOVIS* INFECTION IN BADGERS *MELES MELES* AND CATTLE IN FOUR AREAS IN IRELAND

Gabrielle Kelly (UCD Statistics), Simon More (UCD CVERA), David Williams (UCD Statistics), Guy McGrath (UCD CVERA)

An understanding of spatial associations between *M. bovis* in badgers and cattle can contribute to our understanding of disease behaviour and the effectiveness of existing control policies. In this study, we are investigating local spatial associations between *M. bovis* infection in badgers and cattle, using data from the four area project.

WINTER YARD SURVEY

Paddy Sleeman, John Davenport, Anthony Fitzgerald (UCC Zoology)

Visits by infected badgers to yards where cattle are housed in winter may provide opportunities for transmission of tuberculosis to cattle. Using winter survey periods in 2005/06 and 2006/07, this study has sought to quantify the badger activity in cattle yards in Co. Cork.

QUANTIFYING BADGER EXPOSURE AND THE RISK OF BOVINE TUBERCULOSIS FOR CATTLE HERDS IN COUNTY KILKENNY, IRELAND

Olea-Popelka, F.J., Phelan, J., White, P.W., McGrath, G., Collins, J.D., O'Keeffe, J., Duggan, M., Collins, D.M., Kelton, D.F., Berke, O., More, S.J., Martin, S.W.

Preventive Veterinary Medicine 75 (2006), 34–36.

The objectives of the study were to quantify the levels of badger exposure for cattle and to test the hypothesis that increased badger exposure does not increase the risk of bovine tuberculosis in a herd. Information that became available from the targeted removal of badgers over the study period, and from a badger-removal project in County Kilkenny during 1996–1999, was used. 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 barnyard during winter, was used to build an exposure coefficient to quantify the amount of badger exposure that cattle encountered either on pasture or in the barn. The study design was a matched case-control study in which the control herds were selected using incidence density sampling. During the 4-year study period, 543 badgers were removed and of these 96 badgers were classified as tuberculosis positive; 96 TB herd breakdowns occurred. There was a significant association between case herds and having a higher badger sett exposure coefficient during 1996–1998. No significant association between case herds and having a higher exposure coefficient based on the number of badgers, or the number of tuberculous badgers, during September 1997–December 1999 was found.

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“We report an association between sett numbers and risk of a TB breakdown, (however,) this has not elaborated possible routes of transmission.”

b. The implications of badger control activities

DOES REACTIVE BADGER CULLING LEAD TO AN INCREASE IN TUBERCULOSIS IN CATTLE?

More, S.J., Clegg, T.A., McGrath, G., Collins, J.D., Corner, L.A.L., Gormley, E.

Veterinary Record 161 (2007), 208-209.

Badgers play an important role in the epidemiology of bovine tuberculosis in Ireland and the UK, and a range of control measures are in place or have been under consideration, including badger culling. In the UK, there is a concern, based principally on results from the random badger culling trial (RBCT), that reactive badger culling may be counterproductive, leading to increased TB incidence in associated cattle and the residual badger population. A cascade of adverse events following badger culling has been proposed, whereby badger culling results in substantial changes to the spatial and social organisation and the territorial behaviour of badger populations (these steps are collectively termed 'perturbation'), which in turn lead to increased contact and transmission of infection between badgers, increased contact between cattle and the disturbed badger population, and increased infection risk in associated cattle. In this article, we question aspects of the interpretation of the RBCT data, in particular the biological plausibility of measured effects, the precision of these effects and the timing of biological processes, and of the accuracy of spatial data. It is important that interested policy-makers and the general public are aware of varying perspectives surrounding this topic.

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“It is important that policy-makers and the general public are aware of varying perspectives about the impact of reactive badger culling on tuberculosis in cattle.”

REACTIVE BADGER REMOVAL AND LEVELS OF BOVINE TUBERCULOSIS IN CATTLE HERDS IN CO. LAOIS

Francisco Olea-Popelka (University of Guelph), James O’Keeffe, Paul White, Pat Flanagan (DAFF), Simon More (UCD CVERA), Wayne Martin (University of Guelph)

One of the current constraints to the eradication of bovine tuberculosis in Ireland is the existence of an important wildlife reservoir for *M. bovis*, namely the badger (*Meles meles*). Targeted (or reactive) badger removal has been conducted in Ireland since the early 1990s. The objective of our study is to assess the impact of targeted badger removal on the subsequent levels of TB for herds in one county in Ireland.

THE IMPACT OF TARGETED BADGER REMOVAL ON TUBERCULOSIS IN CATTLE HERDS IN CO. MONAGHAN

Paul White (UCD CVERA), Klaas Frankena (Wageningen University), James O’Keeffe (DAFF), Simon More (UCD CVERA), Mart de Jong (Wageningen University), Wayne Martin (University of Guelph)

Following the establishment of the Wildlife Unit in October 2003, a policy of targeted badger removal in the areas surrounding TB breakdown herds was implemented in the Republic of Ireland. This study will assess the impact of targeted badger removal on the subsequent levels of TB in herds in Co. Monaghan.

THE EFFECT OF VARYING LEVELS OF POPULATION CONTROL ON THE PREVALENCE OF TUBERCULOSIS IN BADGERS IN IRELAND

Leigh Corner (UCD Agriculture, Food Science and Veterinary Medicine), Tracy Clegg, Simon More (UCD CVERA), David Williams (UCD Statistics), Ian O'Boyle, Eamon Costello (DAFF), Paddy Sleeman (UCC Zoology), John Griffin (DAFF)

In Ireland, the role of badgers in the epidemiology of TB in cattle has become increasingly understood. As yet, however, there is limited understanding of TB epidemiology in badgers. In this study, we are examining the effect of varying levels of population control on the prevalence of *M. bovis* infection in Irish badger populations.

AN ASSESSMENT OF INJURY TO BADGERS DUE TO CAPTURE IN STOPPED RESTRAINTS

Denise Murphy (UCD Agriculture, Food Science and Veterinary Medicine), James O'Keeffe (DAFF), Wayne Martin (University of Guelph), Leigh Corner (UCD Agriculture, Food Science and Veterinary Medicine).

In Ireland, stopped restraints have been used routinely by DAFF to capture badgers for removal as part of the interim strategy to control bovine tuberculosis. The aim of this study was to determine the frequency and severity of physical injuries occurring when badgers were captured using stopped restraints. Badgers were examined from removal operations carried out by DAFF from October to December 2005 and from May to June 2006. The results showed that the severity of physical injury to badgers in association with the use of stopped restraints was low.

TESTING THE EFFECTIVENESS OF BARRIERS DURING THE IRISH FOUR AREA STUDY

Paddy Sleeman, John Davenport (UCC Zoology), Simon More, Tracy Clegg (UCD CVERA), John Griffin, Ian O'Boyle (DAFF)

Knowledge of badger movements will contribute to our understanding of the dynamics of bovine tuberculosis in badgers and cattle. Using data from the four area study, we are examining the effectiveness of various barriers to badger movements.

DESCRIPTION OF A MEDIUM TERM NATIONAL STRATEGY TOWARD ERADICATION OF TUBERCULOSIS IN CATTLE IN IRELAND

James O'Keeffe (DAFF)

Proceedings of the 11th Symposium of the International Society for Veterinary Epidemiology and Economics (ISVEE), Cairns, Australia, p502.

A compulsory national bovine tuberculosis eradication programme has been operating in the Republic of Ireland since 1959. Substantial progress was achieved in the early decades, but since the mid '70s there has been no improvement despite the continuing application of a very intensive national tuberculin testing programme. Geographical information systems techniques including kernelling have been used to identify areas of the country 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. These areas and the techniques used to delineate them are described. A strategy based on reducing the local densities of badgers, weighted toward more intensive removals in the areas of the country defined as "chronic" is described. In the short term, this will result in lowering the risk of cattle herds becoming infected with TB from 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.

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“The eradication of tuberculosis in cattle is contingent on reducing levels of tuberculosis in the national badger population.”

BADGER TUBERCULOSIS VACCINE

a. Vaccine development (studies with captive badgers)

BADGER TUBERCULOSIS VACCINE

Denise Murphy, Leigh Corner (UCD Agriculture, Food Science and Veterinary Medicine), Eamon Costello (DAFF), Eamonn Gormley (UCD Agriculture, Food Science and Veterinary Medicine)

The research on BCG vaccination against tuberculosis in badgers, consisting of an integrated series of experiments and associated studies, is continuing. Further, a series of studies have been completed with a view to implementing field vaccination of badgers. The BCG vaccine was initially chosen for use based on its availability, low production cost and much experience of its application in domestic and wild animals, and humans. To carry out these studies in a controlled environment, the Badger Research and Observation Centre (BROC) was designed to hold 6-7 small groups of badgers. In parallel with the captive badger experiments, studies have also been undertaken in wild, naturally infected badgers, using badgers removed during culling operations. As part of these studies, we helped develop and assess a range of *in vitro* diagnostic assays based on the immunological responses to challenge with virulent *M. bovis*.

BROC 1: Experimental infection with *M. bovis*

As a first step in development of a vaccine, an infection model was required to generate disease in captive badgers. The objective of BROC 1 was to identify a dose of *M. bovis* which, when delivered by the endobronchial route to badgers, generated a disease profile that mimicked natural disease. The results showed that over a wide range of doses, the endobronchial procedure produced disease that was characteristic of natural disease. See publication below.

BROC 2: Progression of the experimental infection

Having established the utility of the endobronchial route and an effective challenge dose, this study was designed to use the optimal infective dose (derived from BROC 1) and follow the progression of the disease over time. This was important to determine the optimal time to examine the differences in the progression of disease between vaccinates and controls. Following experimental challenge, infection progressed slowly with a uniform result across the badgers studied. The optimal time to examine disease in vaccinates was found to be 12 to 18 weeks after infection.

BROC 3: Establishing proof that BCG induces protection against tuberculosis in badgers

With the infection procedure established, we set out to determine if BCG was protective in badgers and to examine possible routes of vaccination including the subcutaneous and mucosal routes. The results showed that vaccination by either route led to significant protection of vaccinated badgers compared with non-vaccinated controls.

BROC 4: Oral BCG vaccination and protection

To be of practical use in the field delivery of BCG, an oral bait is likely to be the most cost-effective means of delivery. A lipid formulation that protects the live BCG from gastric secretions has been developed by Dr Frank Aldwell (University of Otago, New Zealand). Having demonstrated that BCG generated protection in badgers, and that a mucosal route was highly effective, we wanted to test the efficacy of BCG vaccine delivered by the oral route after challenge by the endobronchial infection procedure. The outcome was that vaccination by the oral route led to significant protection.

BROC 5: Duration of protection following oral BCG vaccination

Having demonstrated that oral delivered BCG could induce protection, we set out to determine if badgers vaccinated by oral routes with a lipid-encapsulated BCG would induce protection that could be detected for up to 12 months. The study is due for completion.

BROC 6: Comparison of protection of badgers vaccinated with BCG-Pasteur and commercial BCG-Danish vaccine strains

To date, all of our studies have been conducted using the BCG-Pasteur strain. However, currently the only BCG vaccine strain produced and registered in the EU is the BCG-Danish strain manufactured by Statens Serum Institut (Denmark). There is now an international consensus that the vaccine submitted for registration as veterinary medicine will use this strain. In this study, we compared oral vaccination with the two BCG strains by feeding badgers with the BCG strains encapsulated in a semi-solid lipid matrix that was prepared specifically for this purpose by the collaborating laboratory in New Zealand (Dr Frank Aldwell, University of Otago). The results indicate that both vaccines generated high levels of protective immunity. There are no significant differences between BCG-Danish and BCG-Pasteur strains.

BROC 7: The protective efficacy of BCG-Danish in badgers against a low dose challenge with *M. bovis*

We have established that the BCG vaccine can protect badgers against experimentally induced disease when the challenge protocol used a high dose of *M. bovis* (10^4 cfu). The purpose of the current study is to determine what effect vaccination will have on the experimental disease against a realistic low challenge dose and over a longer timescale. Badgers have been vaccinated with BCG-Danish strain, encapsulated in a semi-solid matrix. A control group remains non-vaccinated. Twelve weeks after vaccination, the badgers were challenged by the endobronchial route with a low dose of *M. bovis* (10^2 cfu). At 52 weeks post-challenge, the badgers will be euthanased and protection assessed by pathology and culture. The data generated will be used to further our knowledge of the pathogenesis of tuberculosis in the badger and how vaccination alters the progression of the disease.

EXPERIMENTAL TUBERCULOSIS IN THE EUROPEAN BADGER (*MELES MELES*) AFTER ENDOBRONCHIAL INOCULATION OF *MYCOBACTERIUM BOVIS*: I. PATHOLOGY AND BACTERIOLOGY

Corner, L.A., Costello, E., Lesellier, S., O'Meara, D., Sleeman, D.P., Gormley, E.

Research in Veterinary Science 83 (2007), 53-62.

The aim was to develop an endobronchial infection procedure for the study of *Mycobacterium bovis* infection in badgers. The badgers were anaesthetised and a cannula was passed *per os* to the tracheal bifurcation. When in place, 1 ml of *M. bovis* suspension was inoculated. Three concentrations of *M. bovis* suspension were used; <10 colony forming units (cfu), approximately 10^2 cfu and approximately 3×10^3 cfu. The badgers were examined at three weekly intervals for clinical signs of disease and a tracheal aspirate was collected at each examination. The badgers were euthanased 17 weeks post infection (pi) and at the *post mortem* examination a wide range of tissues were examined for gross and histopathological lesions of tuberculosis and cultured for *M. bovis*. A sample of bronchial alveolar lavage (BAL) fluid was collected at post mortem for culture. At *post mortem* examination 17 weeks after infection, gross and histopathological lesions of tuberculosis were observed in all badgers inoculated with the high and medium dose and in 1 of 3 animals inoculated with the low dose. *M. bovis* was recovered from all inoculated badgers. Infection in the high dose group was more widely disseminated than in the other groups. The number of sites with gross and histopathological lesions increased with an increasing dose of *M. bovis*. All tracheal aspirates were negative on culture and only one BAL, collected from a badger of the high dose group, was positive on culture. No clinical signs due to the experimental infection were observed. The endobronchial route of inoculation is an effective route for establishing experimental infection, and could be used for studies of tuberculosis pathogenesis, immunology of *M. bovis* infection in badgers and for challenging badgers in vaccine protection studies. Badgers appeared to be very susceptible to infection by this procedure even with a dose of < 10 cfu but appear to control and limit the resulting infection.

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“Badgers appear to be very susceptible to *M. bovis* infection, but appear to control and limit the resulting infection.”

b. Vaccine evaluation (field studies)

THE VACCINE FIELD TRIAL

Denise Murphy, Leigh Corner (UCD Agriculture, Food Science and Veterinary Medicine), Eamon Costello (DAFF), Eamonn Gormley (UCD Agriculture, Food Science and Veterinary Medicine)

The results from the captive badgers studies suggest that the BCG might be an ideal vaccine for use in wild badger populations. However, such studies cannot show that BCG vaccine will be protective in free-ranging badgers or provide an estimate of vaccine efficacy. This can only be determined in a field trial. Such a trial is planned with a large population of badgers and will be carried out in a defined area over three years. Badgers will receive vaccine or placebo, sequentially establishing a 50% vaccine coverage. Throughout the trial, estimates of changing incidence will be made from the measurements of individual immune responses. At the end of the study, the area will be depopulated and all badgers examined for tuberculosis by culture. The vaccine efficacy will be estimated from a comparison of the number of infected badgers in the vaccine group with the non-vaccinated control group. The results and experience gained from the field trial will facilitate the development of strategies for introduction of vaccination into the national programme.

c. Supporting work

DEVELOPMENT OF BADGER IMMUNODIAGNOSTICS

Sandrine Lesellier, Eamonn Gormley (UCD Agriculture, Food Science and Veterinary Medicine)

With the development of a vaccine for use in badgers, accurate tests will be required for tuberculosis surveillance in badger populations and to monitor the effect of vaccination. As part of the captive badger vaccine studies, we have tested a range of novel immunodiagnostic tests, developed through collaborations with VLA (Weybridge, UK) and shown that highly sensitive diagnostic tests are possible for badgers. In addition, with recognition of high specificity antigens it appears that the development of differential diagnosis is possible; such a test would be invaluable for use in association with a badger vaccine strategy.



Laboratory testing plays an important role in the diagnosis of *Mycobacterium bovis*

ANTIGEN SPECIFIC IMMUNOLOGICAL RESPONSES OF BADGERS (*MELES MELES*) EXPERIMENTALLY INFECTED WITH *MYCOBACTERIUM BOVIS*

Sandrine Lesellier, Leigh Corner (UCD Agriculture, Food Science and Veterinary Medicine), Eamon Costello (DAFF), Eamonn Gormley (UCD Agriculture, Food Science and Veterinary Medicine) and others

European badgers (*Meles meles*) are considered to be an important reservoir of infection for *M. bovis* and are implicated in the transmission of tuberculosis to cattle in Ireland and the United Kingdom. Accurate tests are required for tuberculosis surveillance in badger populations and to provide a basis for the development of strategies, including vaccination, to reduce the incidence of the infection. In this study, we have developed an endobronchial *M. bovis* infection model in badgers in which we measured cell-mediated immune and serological responses for up to 24 weeks post-infection.

ADVERSE REACTIONS TO BCG VACCINATION AGAINST TUBERCULOSIS

Denise Murphy, Leigh Corner, Eamonn Gormley (UCD Agriculture, Food Science and Veterinary Medicine)

The *M. bovis* strain, bacille Calmette-Guérin (BCG) is one of the most widely used human vaccines and remains one of the safest vaccines available. It has been used in human populations for over 80 years and 100 million children receive the vaccine annually. It has also been employed extensively for vaccine studies in laboratory animal hosts and is currently being developed for use in a variety of livestock and wild animals, including badgers. With continued use of the BCG against tuberculosis in different host species, the risk factors associated with adverse reactions may need to be re-evaluated. In this study we are reviewing the development of adverse reactions to BCG vaccination as reported in humans as well as in a variety of laboratory animals, livestock and wildlife.

INFECTION DYNAMICS AND EFFECTIVE CONTROL STRATEGIES OF TUBERCULOSIS IN BADGERS AND CATTLE OF IRELAND

Inma Aznar (UCD CVERA), Mart de Jong, Klaas Frankena (Wageningen University), Simon More (UCD CVERA)

In Ireland, the control of bovine tuberculosis, leading ultimately to eradication, cannot be achieved until badger-to-cattle transmission is effectively addressed. In this study, we seek to develop a mathematical model of *M. bovis* transmission in cattle and badgers. Ultimately, the model will allow us to assess the potential impact of various interventions strategies on the prevalence of infection in both badgers and cattle.

EVALUATION OF THE ANAESTHETIC EFFECTS OF COMBINATIONS OF KETAMINE, MEDETOMIDINE, ROMIFIDINE AND BUTORPHANOL IN EUROPEAN BADGERS (*MELES MELES*)

Davison, K.E., Hughes, L., Gormley, E., Lesellier, S., Costello, E., Corner, L.A.L.

Veterinary Anaesthesia and Analgesia 34 (2007), 394–402.

The objective of the study was to evaluate the effects of three anaesthetic combinations in adult European badgers (*Meles meles*). The badgers were each anaesthetized by intramuscular injection using the three techniques assigned in random order: romifidine 0.18 mg/kg, ketamine 10 mg/kg and butorphanol 0.1 mg/kg (RKB); medetomidine 0.1 mg/kg, ketamine 9 mg/kg and butorphanol 0.1 mg/kg (MKB); and medetomidine 0.1 mg/kg and ketamine 10 mg/kg (MK). Initial drug doses were calculated based on a body mass of 10 kg. Additional anaesthetic requirements, time to drug effect, duration of action and recovery from anaesthesia were recorded. Heart rate and rhythm, respiratory rate and rhythm, rectal and subcutaneous microchip temperature and oxygen saturation were recorded every 5 minutes. Depth of anaesthesia was assessed using: muscle tone; palpebral and pedal reflexes; and tongue relaxation at these time points. Blood samples and a tracheal aspirate were obtained under anaesthesia. Atipamezole was administered if the badger had not recovered within 60 minutes. Parametric data were analysed using ANOVA for repeated measures, and nonparametric data using Friedman's, and Cochran's Q tests; $p < 0.05$ was considered significant. The result showed that all combinations produced good or excellent muscle relaxation throughout the anaesthetic period. RKB had the shortest duration of anaesthesia (16.8 minutes compared with MKB 25.9 minutes and MK 25.5 minutes) and antagonism was not required. RKB depressed respiratory rate less than MK and MKB. There was no significant difference between techniques for heart rate and rhythm. All combinations provided anaesthetic conditions suitable for sampling and identification procedures in adult badgers. The RKB protocol provided a significantly shorter period of anaesthesia when compared with the combinations containing medetomidine.

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“All combinations provided anaesthetic conditions suitable for sampling and identification procedures in adult badgers.”

IMMUNOLOGICAL RESPONSES AND PROTECTIVE IMMUNITY AGAINST TUBERCULOSIS CONFERRED BY VACCINATION OF BALB/C MICE WITH THE ATTENUATED *MYCOBACTERIUM TUBERCULOSIS* (PHOP) SO2 STRAIN

Aguilar, D., Infante, E., Martin, C., Gormley, E., Gicquel, B., Hernandez Pando, R.

Clinical and Experimental Immunology 147 (2007), 330–338.

The *Mycobacterium tuberculosis* *phoP* mutant strain SO2 has been shown previously to be more attenuated than *Mycobacterium bovis* bacillus Calmette-Guérin (BCG) and confers protective immunity against tuberculosis in mice and guinea pig models. In this study we have investigated the survival and immunological responses of Balb/c mice infected with the *M. tuberculosis* SO2 strain. All Balb/c mice survived intratracheal infection with *M. tuberculosis* SO2 strain under conditions where all the mice infected with the parental *M. tuberculosis* MT103 had died after 9 weeks. Infection of Balb/c mice with *M. tuberculosis* SO2 was associated with comparatively lower levels of interferon (IFN)-gamma, interleukin (IL)-4 and tumour necrosis factor (TNF)-alpha and higher levels of inducible nitric oxide synthase (iNOS) during the late stage of infection, when compared with *M. tuberculosis* MT103 infection. The delayed-type hypersensitivity (DTH) response against *M. tuberculosis* culture filtrates was similar in mice infected with either the *M. tuberculosis* *phoP* SO2 strain or *M. tuberculosis* MT103. The protective efficacy of *M. tuberculosis* SO2 was compared with *M. bovis* BCG when delivered subcutaneously to groups of Balb/c mice. Following intratracheal challenge with *M. tuberculosis* H37Rv, protection was generated by 60 days post-challenge in mice vaccinated with either vaccine. At day 120 post-challenge the levels of protection were still significantly greater when compared with the non-vaccinated control group. The levels of protection conferred by vaccination with *M. tuberculosis* SO2 or with *M. bovis* BCG were similar, as measured by granuloma coalescence and pneumonia in addition to growth reduction of *M. tuberculosis* H37Rv.

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“The *Mycobacterium tuberculosis phoP* mutant strain SO2 is more attenuated than *Mycobacterium bovis* bacillus Calmette-Guérin (BCG) and confers protective immunity against tuberculosis in mice and guinea pig models.”

THE LIVE *MYCOBACTERIUM TUBERCULOSIS* *phoP* MUTANT STRAIN IS MORE ATTENUATED THAN BCG AND CONFERS PROTECTIVE IMMUNITY AGAINST TUBERCULOSIS IN MICE AND GUINEA PIGS

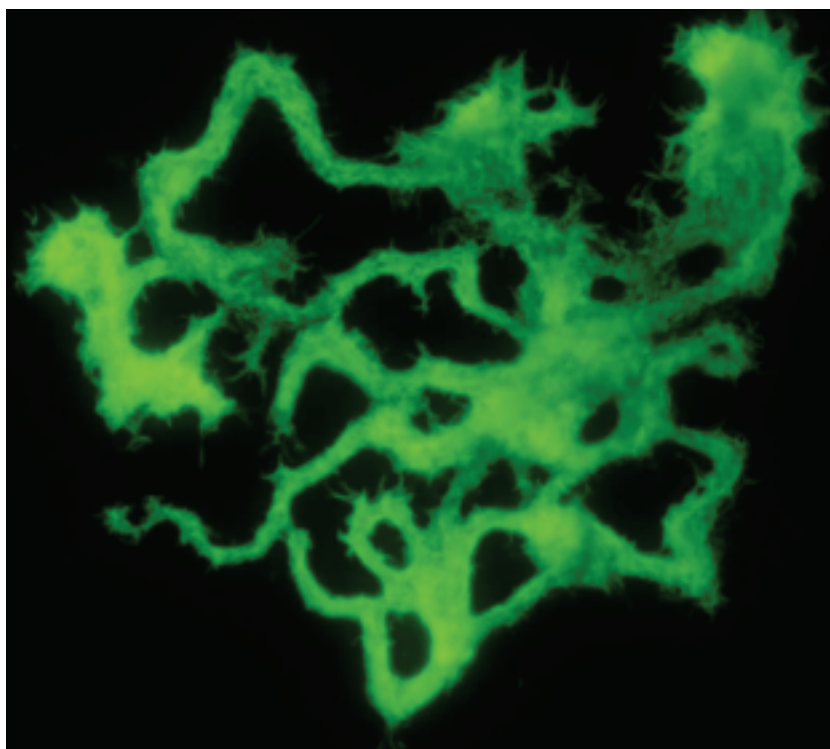
Martin, C., Williams, A., Hernandez-Pando, R., Cardona, P.J., Gormley, E., Bordat, Y., Soto, C.Y., Clark, S.O., Hatch, G.J., Aguilar, D., Ausina, V., Gicquel, B.

Vaccine 24 (2006), 3408-3419.

The *Mycobacterium tuberculosis* *phoP* mutant strain SO2 has previously been shown to have reduced multiplication in mouse macrophages and *in vivo* using the mouse intravenous-infection model. In this study we demonstrate that the *M. tuberculosis* SO2 is highly attenuated when compared with the parental *M. tuberculosis* MT103 strain and also more attenuated than BCG in severe combined immunodeficiency disease (SCID) mice. Complementation of the *M. tuberculosis* SO2 with the wild-type *phoP* gene restored the virulence of the strain in the SCID mice, confirming that the attenuated phenotype is due to the *phoP* mutation. In Balb/c mice subcutaneously vaccinated with either *M. tuberculosis* SO2 or BCG, the proportions of CD4+ and CD8+ populations measured in the spleen were significantly higher in the *M. tuberculosis* SO2 vaccinated group. In addition, the proportion of antigen-stimulated CD4+/CD8+ cells expressing IFN-gamma was significantly higher in the *M. tuberculosis* SO2 vaccinated group when compared with the BCG group. Balb/c mice subcutaneously vaccinated with the *M. tuberculosis* SO2 strain were also protected against intravenous challenge with *M. tuberculosis* H37Rv at levels comparable to mice vaccinated with BCG, as measured by reduced bacterial counts in lung and spleens. Guinea pigs subcutaneously vaccinated with the *M. tuberculosis* SO2 strain were protected against aerosol challenge with *M. tuberculosis* H37Rv delivered at different doses. A high dose aerosol challenge of *M. tuberculosis* SO2 vaccinated guinea pigs resulted in superior levels of protection when compared with BCG vaccination, as measured by guinea pig survival and reduction in disease severity in the lung.

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“A high dose aerosol challenge of *M. tuberculosis* SO2 vaccinated guinea pigs resulted in superior levels of protection when compared with BCG vaccination.”



Confocal fluorescence microscopy of auramine stained *M. tuberculosis* bacilli.
Photo courtesy of Dr Carlos Martin, University of Zaragoza, Spain

TUBERCULOSIS IN OTHER SPECIES

BOVINE TUBERCULOSIS IN ALPACA IN IRELAND

Eoin Ryan (UCD Agriculture, Food Science and Veterinary Medicine), Dónal Connolly (Gort, Co. Galway), PJ Dwyer, John Fagan, Eamon Costello, Martin Hayes, Ascinta Kilroy (DAFF), Simon More (UCD CVERA)

Tuberculosis, due to infection with *M. bovis*, was diagnosed in a flock of alpaca in Ireland in 2004. In this study, we describe the case, including an assessment of infection risk for alpaca farmed in areas where TB is endemic, the origin of the infection, the potential for alpaca-to-alpaca transmission, and appropriate control measures, including the efficacy of predictor tests.



An alpaca in low body condition infected with *Mycobacterium bovis*.
Courtesy of the Irish Veterinary Journal



Tuberculous lesions evident on the lung surface at *post mortem*.
Courtesy of the Irish Veterinary Journal

CONTROL OF *MYCOBACTERIUM BOVIS* INFECTION IN TWO SIKA DEER HERDS IN IRELAND

Tom Partridge, John Egan, Dónal Toolan (DAFF), Simon More (UCD CVERA)

In a number of countries, tuberculosis due to *M. bovis* is a significant health problem of captive deer. In this study, we describe outbreaks of bovine tuberculosis in sika deer (*Cervus nippon*) on two farms in Ireland and the methods used to control the disease.

TUBERCULOSIS TESTING IN DEER

Simon More (UCD CVERA) with other members of the working group (tuberculosis in deer) of the Animal Health and Welfare panel of the European Food Safety Authority (EFSA)

The movement of live deer is linked with the spread of bovine tuberculosis. Ideally, science-based controls are needed to minimise infection risk during intra-community live animal trade, and importation of live animals from third countries. However, such controls are constrained by problems associated with the accuracy of the intradermal skin test in live deer. Given this context, the EFSA working group has sought to address each of the following queries from DG Sanco:

- the suitability of the existing TB tests for deer for the purpose of granting official TB-free status in the framework of Directive 92/65/EEC;
- the modalities for the validation of a TB test for deer; and
- a definition, including options for possible testing regimes giving sufficient guarantees for a animal/holding/region to be qualified/maintained/regained as officially free from TB infection in deer.

ESTIMATING TB PREVALENCE IN DEER

Francisco Olea-Popelka (University of Guelph), James O'Keeffe (DAFF)

The role of wild deer in the epidemiology of bovine tuberculosis in Ireland remains uncertain. This study was conducted to estimate the sample size required to calculate the prevalence of tuberculosis in deer populations in Ireland.



Deer may play a role in the epidemiology of bovine tuberculosis on farmland adjoining areas of forestry

IMPROVED UNDERSTANDING OF DISEASE EPIDEMIOLOGY

a. Why are some herds at higher risk? How can they be managed?

RISK FACTORS FOR DISCLOSURE OF ADDITIONAL TUBERCULOUS CATTLE IN ATTESTED-CLEAR HERDS THAT HAD ONE ANIMAL WITH A CONFIRMED LESION OF TUBERCULOSIS AT SLAUGHTER DURING 2003 IN IRELAND

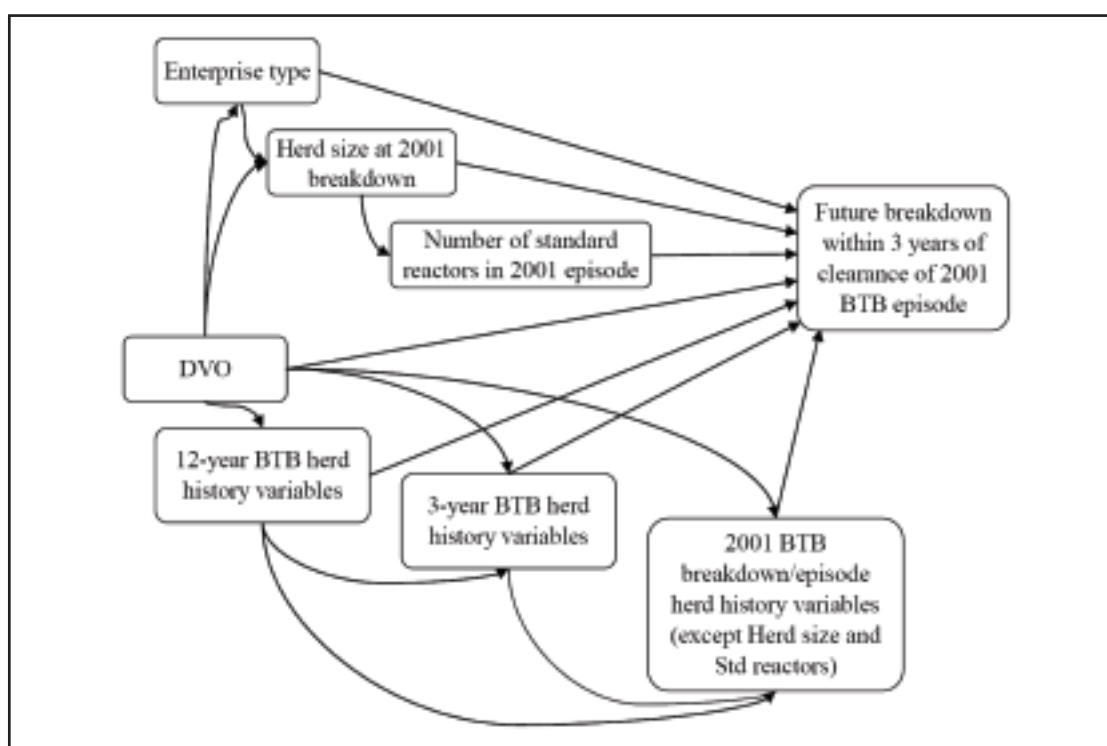
Francisco Olea-Popelka (University of Guelph), Eamon Costello, Paul White (DAFF), Guy McGrath, Dan Collins (UCD CVERA), James O'Keeffe (DAFF), David Kelton, Olaf Berke (University of Guelph), Simon More (UCD CVERA), Wayne Martin (University of Guelph)

In Ireland, TB herd breakdowns are first detected using field tuberculin testing (detecting 73.9% of breakdowns during the 12 months from 1 April 2003) and factory surveillance (26.1%). In those breakdowns first detected by factory surveillance, further standard reactor(s) are found during subsequent tuberculin testing in about 20% of herds; the rest test clear or have minimal TB problems. In this study, we will attempt to identify factors that relate to this difference in outcome.

DISEASE HISTORY PREDICTORS OF BOVINE TUBERCULOSIS BREAKDOWNS IN IRISH CATTLE HERDS

Dianna Wolfe, Olaf Berke, David Kelton (University of Guelph), Paul White (DAFF), James O'Keeffe (DAFF), Wayne Martin (University of Guelph)

In the Republic of Ireland, 20,000 to 50,000 cattle have been slaughtered annually due to bovine tuberculosis over the past 4 decades. Since the inception of the eradication programme, it has been noted that the risk of a TB breakdown is higher in some herds than others. In an effort to focus resources on problem herds, a herd classification scheme has been developed by DAFF based on the number of test-positive animals and the number of slaughtered animals with lesions occurring in a herd over a TB episode. The current study, a retrospective cohort study, was undertaken to investigate risk factors for future TB breakdowns in Irish cattle herds, with a goal of refining this herd risk scoring system.



Causal diagramme of risk factors (relating to the disease history of a herd) associated with a TB recurrence within three years of clearance following a TB episode in Ireland during 2001

DESCRIPTIVE ANALYSIS OF HERDS DISCLOSING MULTIPLE ANIMALS WITH A BOVINE TUBERCULOSIS LESION AT SLAUGHTER DURING YEAR 2003 IN IRELAND

Francisco Olea-Popelka (University of Guelph), Eamon Costello, Paul White (DAFF), Guy McGrath, Dan Collins (UCD CVERA), James O'Keeffe (DAFF), David Kelton, Olaf Berke (University of Guelph), Simon More (UCD CVERA), Wayne Martin (University of Guelph)

This study involved all Irish cattle herds considered 'clear' of bovine tuberculosis but having multiple animals with a tuberculous lesion at slaughter during 2003. In this study, we conducted a descriptive analysis of selected risk factors with the potential to impact upon the result of the herd test immediately after the tuberculous lesion animals were found.

A CASE CONTROL STUDY OF TEMPORAL AND SPATIAL RISK FACTORS ASSOCIATED WITH BOVINE TUBERCULOSIS BREAKDOWN HERDS IN IRISH CATTLE HERDS IN 2006

Paul White (UCD CVERA), Klaas Frankena (Wageningen University), James O'Keeffe (DAFF) Simon More (UCD CVERA), Mart de Jong (Wageningen University)

The temporal and spatial clustering patterns of tuberculin reactor disclosure are well recognised in Ireland. Using logistic regression techniques, this paper aims to determine the extent to which the presence or absence of a TB episode in year 2006 could have been predicted, based on previous TB history of the index herd over the years 1989-2005 and the TB history contiguous herds over the years 1989-2006.

b. How important is contiguous spread?

A CASE STUDY OF BOVINE TUBERCULOSIS IN AN AREA OF COUNTY DONEGAL, IRELAND

Olea-Popelka, F.J., Butler, D., Lavin, D., McGrath, G., O'Keeffe, J., Kelton, D., Berke, O., More, S., Martin, S.

Irish Veterinary Journal 59 (2006), 683-690.

We performed a descriptive analysis to investigate the potential risk factors that might have contributed to the increased incidence of bovine tuberculosis (TB) 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 TB during the FAP; 10 of these herds were restricted twice, resulting in a total of 82 TB breakdowns. During the first four years of the FAP, the number of TB 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 TB 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 TB breakdowns during the fifth year most likely occurred because of the recrudescence of infection, herd-to-herd transmission, and to a lesser extent 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 TB in herds is a problem that cannot be addressed successfully by dedicating our efforts to the elimination of single risk factors alone. 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.

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“TB in herds is a problem that cannot be addressed successfully by dedicating our 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.”

ESTIMATION OF THE BETWEEN-HERD REPRODUCTION RATIO FOR CONTIGUOUS SPREAD OF BOVINE TUBERCULOSIS

Paul White (UCD CVERA), Klaas Frankena (Wageningen University), James O'Keeffe (DAFF), Simon More (UCD CVERA), Mart de Jong (Wageningen University), Wayne Martin (University of Guelph)

The aim of any eradication programme is to bring the reproductive ratio for the disease below 1. Tuberculin testing data is available from the Animal Health Computer System (AHCS) database for years 1989 to 2007. Together with contiguous data from the Land Parcel Information System, the data will be used for the analysis of possible transmission pathways for bovine tuberculosis between neighbouring herds following initial infection. After back-calculation to determine the sequence of infection between herds, the likelihood of between-herd transmission will be evaluated and an overall reproduction ratio calculated for contiguous spread.

c. How important is introduced infection?

THE RISK OF BOVINE TUBERCULOSIS IN CATTLE PURCHASED FROM HERDS WITH AND WITHOUT A RECENT HISTORY OF BOVINE TUBERCULOSIS IN IRELAND

Dianna Wolfe (University of Guelph), James O'Keeffe, Paul White (DAFF), David Kelton, Olaf Berke, Wayne Martin (University of Guelph)

Previous herd-level epidemiological investigation has demonstrated that some 10% of herds that become derestricted from a TB episode will experience a subsequent TB breakdown on the follow-up test 6 months post-derestriction. In part, this may be because these herds were never truly clear of TB on the date of derestriction, and some animals in the herd may still be infected. Subsequently, these herds may unknowingly be selling *M. bovis*-infected animals. The objective of this study was to test the hypothesis that the odds of testing positive for TB were not significantly different for cattle sold from dairy herds with a recent history of TB than for cattle sold from dairy herds without a recent history of TB. Evidence of a significant difference in the odds of TB in these two groups of animals may indicate that a review of policy governing trade restrictions and pre-movement testing should be considered.

PREDICTORS FOR HERD-TO-HERD MOVEMENT AMONG CATTLE BORN IN IRELAND DURING 2005

Paul White (UCD CVERA), Klaas Frankena (Wageningen University), James O'Keeffe (DAFF), Simon More (UCD CVERA), Mart de Jong (Wageningen University), Wayne Martin (University of Guelph)

The relatively high levels of between-herd movement of cattle within Ireland may pose a risk for transmission of bovine tuberculosis between herds. This study will consist of a survival analysis of a 1% sample of animals born in year 2002. The subsequent between-herd movements, and slaughter events of this population will be monitored over the period from birth up to the end of 2005. Birth data will be derived from the Cattle Movement Monitoring System (CMMS) database, including birth registration and movement/exit data. While animals may move more than once in their lifetime, this study focuses on only the first animal movement during the period from birth up to the end of 2005. The purpose of the study is to identify predictors for this movement based on animal, herd and regional characteristics.

CONTRIBUTING TO POLICY OPTIONS

a. Programme management

TUBERCULOSIS IN CATTLE: STRATEGIC PLANNING FOR THE FUTURE

Collins, J.D.

Veterinary Microbiology 112 (2006), 369-381.

In the later stages of eradication of tuberculosis in cattle, there is a need to take account of the fact that *Mycobacterium bovis* infection in cattle presents, not as cases of clinical disease but most commonly as apparently healthy animals showing an immunological response to tuberculin. This is an entirely different scenario to that seen when national eradication programmes were first devised, at a time when the protection of public health rather than animal health was the prime motivation. In countries with active programmes to eradicate bovine tuberculosis, it is critical for the programme's success that account is taken of this redefinition of tuberculosis, side by side with changes in modern animal production systems and their impact on the transmission of *M. bovis*. This paper highlights factors critical to the success of a national eradication programme, including a clear identification of the goals, of the policies that guide actions, and of the sequences of actions that are required within the programme to accomplish these goals. Experience has illustrated the adverse effects of compromise on outcome when the application of fundamental principles of disease control such as sound animal management, removal of known sources of infection, early diagnosis, quarantine, movement control and environmental hygiene are less than enthusiastically promoted and applied. The reality is that where these principles are applied in a sustained manner, the outcome is more likely to be successful. Therein lies the challenge for the risk manager.

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“Experience has illustrated the adverse effects when the fundamental principles of disease control are less than enthusiastically promoted and applied.”

THE TUBERCULOSIS ERADICATION PROGRAMME IN IRELAND: A REVIEW OF SCIENTIFIC AND POLICY ADVANCES SINCE 1988

More, S.J., Good, M.

Veterinary Microbiology 112 (2006), 239-251.

A national programme to eradicate bovine tuberculosis commenced in Ireland in 1954. During the last 15-20 years, research has been conducted to address gaps in knowledge of disease epidemiology, to objectively evaluate alternative strategy options, and to critically assess the implementation of disease control strategies. This paper provides a review of scientific and policy advances in Ireland since 1988, relevant to the tuberculosis eradication programme in Ireland. There have been substantial advances in knowledge of aspects of disease epidemiology, relating to cattle-to-cattle transmission, the role of wildlife, transmission of infection from wildlife and methods to minimise wildlife-to-cattle transmission. Further, scientific advances have been made both in the detection and management of infected herds. With respect to policy, the paper describes current policy and policy advances in both the detection and management of infected herds, as well as current strategies to prevent herd breakdowns. The Irish programme is a useful example of science-informed policy in a national context.

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“The Irish programme is a practical example of science-informed policy in a national context.”

PROGRESS IN IRELAND TOWARDS THE ERADICATION OF BOVINE TUBERCULOSIS

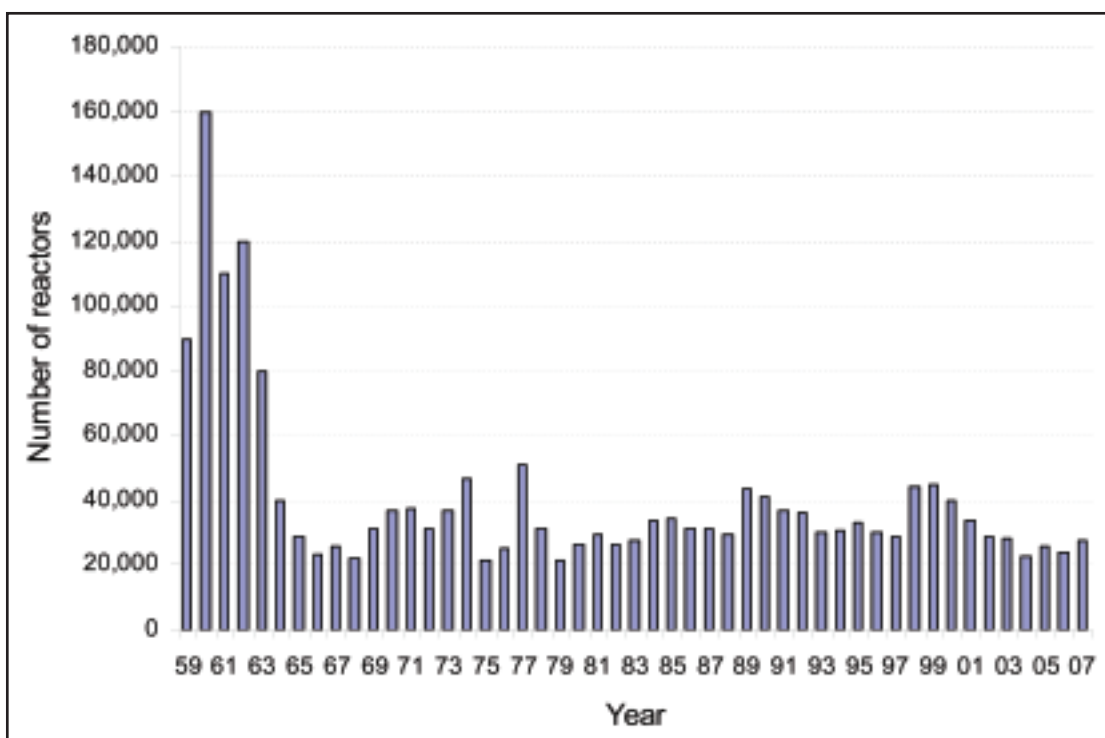
More, S.J.

UK Vet Livestock 12 (2007), 60-63.

There has been a national bovine tuberculosis eradication programme in Ireland since 1954. Initial progress was rapid, but has subsequently stalled despite the implementation of each of the accepted elements of disease control. Based on results from the east Offaly and four area projects, there is now conclusive evidence that wildlife (specifically transmission of infection from badgers to cattle) are a key constraint to disease eradication in Ireland, with cattle-to-cattle transmission of relatively lesser importance. Ireland is currently implementing a comprehensive strategy to address this constraint. In the short-term, a national programme of wildlife control has been implemented in areas of high disease prevalence, in combination with a broad range of other measures. In the longer term, Ireland is committed to the development of an effective badger vaccine and the implementation of a strategic programme of badger vaccination.

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“The programme is underpinned by a comprehensive research programme to understand and address constraints to eradication.”



The number of TB reactors detected in Ireland each year between 1959 and 2007

QUANTIFICATION OF THE CONTRIBUTION OF RISK FACTORS FROM VARIOUS SOURCES, TO CALCULATE AN OVERALL REPRODUCTION RATIO (R_0) FOR BOVINE TUBERCULOSIS INFECTION

Paul White (UCD CVERA), Klaas Frankena (Wageningen University), James O’Keeffe (DAFF), Simon More (UCD CVERA), Mart de Jong (Wageningen University), Wayne Martin (University of Guelph)

This project aims to quantify the contribution of several factors to the persistence of bovine tuberculosis under Irish cattle husbandry conditions. Key factors are the effectiveness of the (existing) test and cull programme, the role of animal trade, the contribution of wildlife (badgers) and the role of contiguity. When the relative importance of these factors has been quantified, several alternative eradication scenarios can be evaluated to assess their potential in TB eradication under Irish conditions by means of a between-herd transmission model.

IMPROVED STATISTICAL MEASURES FOR TB SURVEILLANCE AND CONTROL

Isabella Higgins, Simon More, Tracy Clegg (UCD CVERA), Paul White (DAFF)

Proceedings of the 11th Symposium of the International Society for Veterinary Epidemiology and Economics (ISVEE), Cairns, Australia, p814.

Objective measures of progress are a critical component of any effective disease eradication programme. As part of a tuberculosis eradication programme, it is important that these measures are based on herd as the unit of interest. Also, they should clearly distinguish the results of surveillance and control activities, and use clearly defined case definitions. In Ireland, new statistical measures were developed to assist with decision-making, both locally and at a national level. Measures relating to surveillance and control activities were used, after creating an 'episode' file which defined periods when each herd was (and was not) restricted due to tuberculosis. During 2005, 94.7% of eligible herds remained disease-free during the year. There was minimal correlation between duration of restriction (days) and herd incidence (Pearson's correlation = 0.297, $p = 0.111$). Based on herds restricted on 31 December 2005, there was an average of 5.7 reactors per restriction, with a single reactor detected in 41.2% of restrictions. These herd-level measures effectively partition activities relating to detection of new cases (surveillance) and the resolution of cases following detection (control). They also support earlier findings about herd-level risk factors for herd TB breakdowns in Ireland. Further work is ongoing.

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POTENTIAL INFECTION-CONTROL BENEFIT FOR IRELAND FROM PRE-MOVEMENT TESTING OF CATTLE FOR TUBERCULOSIS

Tracy Clegg, Simon More, Isabella Higgins (UCD CVERA), Margaret Good, Martin Blake (DAFF), David Williams (UCD Statistics)

There is now only limited cattle-to-cattle transmission of bovine tuberculosis in Ireland, as a direct result of a comprehensive national control programme. Additional strategies may help to limit this further. One such option is the use of pre-movement testing for bovine tuberculosis, a strategy that had previously been discontinued in Ireland in 1996. This study seeks to determine the number of restrictions that could be attributed to the movement of infected animals; describe movement events following de-restriction of a herd; estimate the proportion of animals infected at the time of de-restriction; identify high-risk movements (those most likely to involve infected animals) and determine the potential yield of a pre-movement test.

BOVINE TUBERCULOSIS REACTOR MOVEMENT DATA, 2005

Francisco Olea Popelka (University of Guelph)

The bovine TB reactor movement data for 2005 has been collated. The computer code created for this analysis will allow us to describe, compare and contrast the characteristics of each years TB reactor animals and/or herds. These outputs will be an important addition to the range of measures currently used to describe the progress of the bovine tuberculosis eradication scheme in Ireland.

AN EVALUATION OF THE IRISH SINGLE REACTOR BREAKDOWN PROTOCOL FOR 2005 AND 2006, AND ITS USE AS A MONITOR OF TUBERCULIN TEST PERFORMANCE

Margaret Good, Anthony Duignan (DAFF)

The 'Singleton Protocol' has been developed as part of the TB eradication programme in Ireland. This protocol allows for the early restoration of free trading status to herds where:

- a single positive animal was detected, and
- disease in the herd was not confirmed as infected with *M. bovis* by epidemiological investigation, at *post mortem*, by laboratory examination, or by further test.

The study presents data about the Singleton Protocol from 2005 and 2006, highlighting its potential as a monitor for tuberculin test performance.

b. Improving field surveillance

DIAGNOSIS OF *MYCOBACTERIUM BOVIS* INFECTION IN CATTLE BY USE OF THE GAMMA-INTERFERON (BOVIGAM®) ASSAY

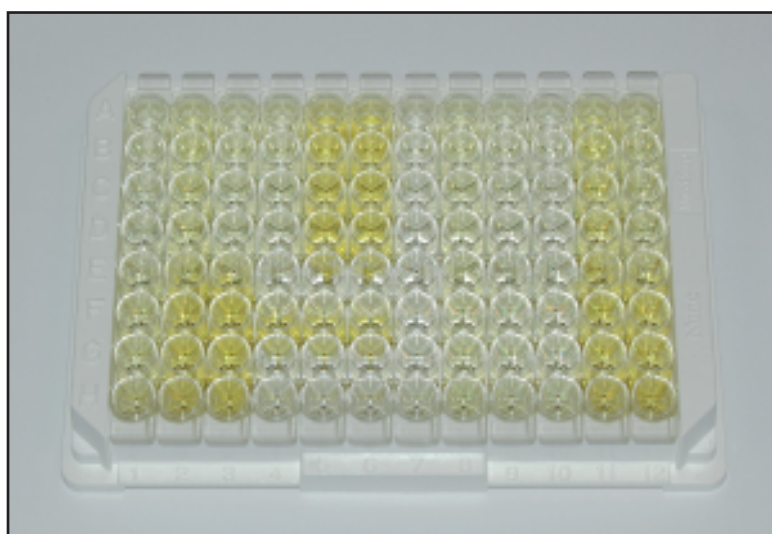
Gormley, E., Doyle, M.B., Fitzsimons, T., McGill, K., Collins, J.D.

Veterinary Microbiology 112 (2006), 171-179.

The strategic use of the gamma-interferon (IFN- γ) assay (Bovigam®) can provide a means for the early identification of *Mycobacterium bovis* infected cattle, thus ensuring their removal from an infected herd. When used in parallel with the tuberculin test, it is capable of identifying infected cattle, which might otherwise not be detected 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 prevalence the benefits to the herd owner directly concerned can be considerable as the assay provides a means of shortening the period of restriction for such herds. This serves to generate confidence among herd owners and other stakeholders that effective schemes, based on sound scientific principles, can be developed to eradicate tuberculosis from infected cattle populations.

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“The strategic use of the gamma-interferon (IFN- γ) assay (Bovigam®) can provide a means for the early identification of *Mycobacterium bovis* infected cattle, thus ensuring their removal from an infected herd.”



IFN- γ ELISA plate assay

QUALITY CONTROL IN TUBERCULIN PRODUCTION AND USAGE

Douwe Bakker (Institute for Animal Science & Health, Lelystad), Margaret Good, Eamon Costello, Anthony Duignan (DAFF), Eamonn Gormley (UCD Veterinary Medicine), Tracy Clegg, Simon More (UCD CVERA)

Quality control is a key, and often problematic, issue during tuberculin production and usage. A series of papers are currently being prepared on issues relating to:

- Tuberculin production;
- Tuberculin potency testing;
- A comparison of tuberculins from different sources and of different potencies, as measured through skin testing and interferon-gamma;
- Variability in tuberculin test results; and
- Quality control of tuberculin testing in the field.

THE COMPARATIVE PERFORMANCE OF THE SINGLE INTRADERMAL COMPARATIVE TUBERCULIN TEST IN IRISH CATTLE, USING TUBERCULIN PPD COMBINATIONS FROM DIFFERENT MANUFACTURERS

Margaret Good, Finbarr Murphy (DAFF), Tracy Clegg, Simon More (UCD CVERA)

Featured in the 'Selected reports' section

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, as currently performed in Ireland.

THE TUBERCULIN TEST – A SAFE MEANS TO TEST A CATTLE POPULATION FOR BOVINE TUBERCULOSIS

Good, M., Higgins, I., Maher, P.

Irish Veterinary Journal 60 (2007), 680-684.

The bovine tuberculosis eradication programme in Ireland relies almost exclusively on the testing of individual animals using the single intradermal comparative tuberculin test (SICTT) to detect TB infected live cattle. Controls under the eradication programme are then applied at herd level. This paper provides a detailed overview of the use of the tuberculin test in Irish cattle.

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TUBERCULIN REGISTRATION

Margaret Good (DAFF)

Tuberculin registration is now required under EU legislation. A range of papers are being completed in support of the registration process, including:

- The tuberculin test – a safe means to test a cattle population for bovine tuberculosis;
- An evaluation of the Irish Single Reactor Breakdown Protocol for 2005 and 2006, and its use as a monitor of tuberculin test performance; and
- Quality control in tuberculin production and usage, which includes an evaluation of the comparative performance of the SICTT in Irish cattle, using tuberculin PPD combinations from different manufacturers.

INCONCLUSIVE TEST REACTORS: FUTURE IMPLICATIONS

Rob Doyle (DAFF)

There is some uncertainty about the future TB status of animals that are inconclusive to the annual tuberculin test. This study will determine future TB risk for a cohort of these animals as identified by the Sligo DVO over an 8 year period. The study will also seek to examine the risk that these animals posed to their herd of residence in that interim period.

GENE EXPRESSION PROFILING OF PERIPHERAL BLOOD MONONUCLEAR CELLS (PBMC) FROM *MYCOBACTERIUM BOVIS* INFECTED CATTLE AFTER *IN VITRO* ANTIGENIC STIMULATION WITH PURIFIED PROTEIN DERIVATIVE OF TUBERCULIN (PPD)

Meade K.G., Gormley, E., Park, S.D., Fitzsimons, T., Rosa, G.J., Costello, E., Keane J., Coussens, P.M., MacHugh, D.E.

Veterinary Immunology and Immunopathology 113 (2006), 73-89.

Microarray analysis of messenger RNA (mRNA) abundance was used to investigate the gene expression program of peripheral blood mononuclear cells (PBMC) from cattle infected with *Mycobacterium bovis*, the causative agent of bovine tuberculosis. An immunospecific bovine microarray platform (BOTL-4) with spot features representing 1336 genes was used for transcriptional profiling of PBMC from six *M. bovis*-infected cattle stimulated *in vitro* with bovine purified protein derivative of tuberculin (PPD-bovine). Cells were harvested at four time points (3 h, 6 h, 12 h and 24 h post-stimulation) and a split-plot design with pooled samples was used for the microarray experiment to compare gene expression between PPD-bovine stimulated PBMC and unstimulated controls for each time point. Statistical analyses of these data revealed 224 genes (approximately 17% of transcripts on the array) differentially expressed between stimulated and unstimulated PBMC across the 24 h time course ($P < 0.05$). Of the 224 genes, 87 genes were significantly upregulated and 137 genes were significantly downregulated in *M. bovis*-infected PBMC stimulated with PPD-bovine across the 24 h time course. However, perturbation of the PBMC transcriptome was most apparent at time points 3 h and 12 h post-stimulation, with 81 and 84 genes differentially expressed, respectively. In addition, a more stringent statistical threshold ($P < 0.01$) revealed 35 genes (approximately 3%) that were differentially expressed across the time course. Real-time quantitative reverse transcription PCR (qRT-PCR) of selected genes validated the microarray results and demonstrated a wide range of differentially expressed genes in PPD-bovine-, PPD-avian- and Concanavalin A (ConA) stimulated PBMC, including the interferon-gamma gene (IFNG), which was upregulated in PBMC stimulated with PPD-bovine (40-fold), PPD-avian (10-fold) and ConA (8-fold) after *in vitro* culture for 12 h. The pattern of expression of these genes in PPD-bovine stimulated PBMC provides the first description of an *M. bovis*-specific signature of infection that may provide insights into the molecular basis of the host response to infection. Although the present study was carried out with mixed PBMC cell populations, it will guide future studies to dissect immune cell-specific gene expression patterns in response to *M. bovis* infection.

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“The pattern of expression of genes in PPD-bovine stimulated PBMC provides the first description of an *M. bovis*-specific signature of infection that may provide insights into the molecular basis of the host response to infection.”

c. Improving factory surveillance

QUANTIFICATION OF THE RELATIVE EFFICIENCY OF FACTORY SURVEILLANCE IN THE DISCLOSURE OF TUBERCULOSIS LESIONS IN ATTESTED IRISH CATTLE

Frankena, K., White, P.W., O'Keefe, J., Costello, E., Martin, S.W., van Grevenhof, I., More, S.J.

Veterinary Record 161 (2007), 679-684.

In Ireland, factory surveillance of cattle for gross lesions is an important supplementary method for detecting herds infected with bovine tuberculosis (TB), and in recent years between 27 and 46 per cent of all new herd breakdowns in any year have been detected by this method. The aim of this study was to determine the relative efficiency of factories in detecting lesions among attested cattle slaughtered during 2003 and 2004. National databases were available on animal slaughter, programmes of tuberculin testing for bovine TB and laboratory confirmation of suspected lesions. Factories were ranked according to their submission risk (number of animals submitted with lesions/number of attested animals killed) and confirmation risk (number of animals with laboratory-confirmed lesions/number of animals submitted with lesions), adjusting for the risk profile of the animals slaughtered, including potential confounding factors such as their age and sex, whether they were purchased or homebred, the test history of their herd, the prevalence of bovine TB in the area and the season of slaughter. Approximately 3.7 million cattle were slaughtered in 42 Irish export-licensed factories during the two years. Complete data were available for 2,374,987 animals from 84,510 attested herds in 2,845 District Electoral Divisions. Samples from 7,398 animals with suspected TB lesions were submitted for laboratory examination; 4,767 (64.4 per cent)

were positive, 2,011 were negative and 620 were inconclusive. The average unadjusted submission risk for all the factories was 22 per 10,000, ranging from 0 to 58 per 10,000. The unadjusted factory confirmation risk (excluding factories that had sent in fewer than 10 lesions) varied between 34.3 and 86.3%. The unadjusted and adjusted submission and confirmation risks were highly correlated, and animal-related factors (including their characteristics and origin) therefore did not contribute to the variations in factory-level submission and confirmation risks.

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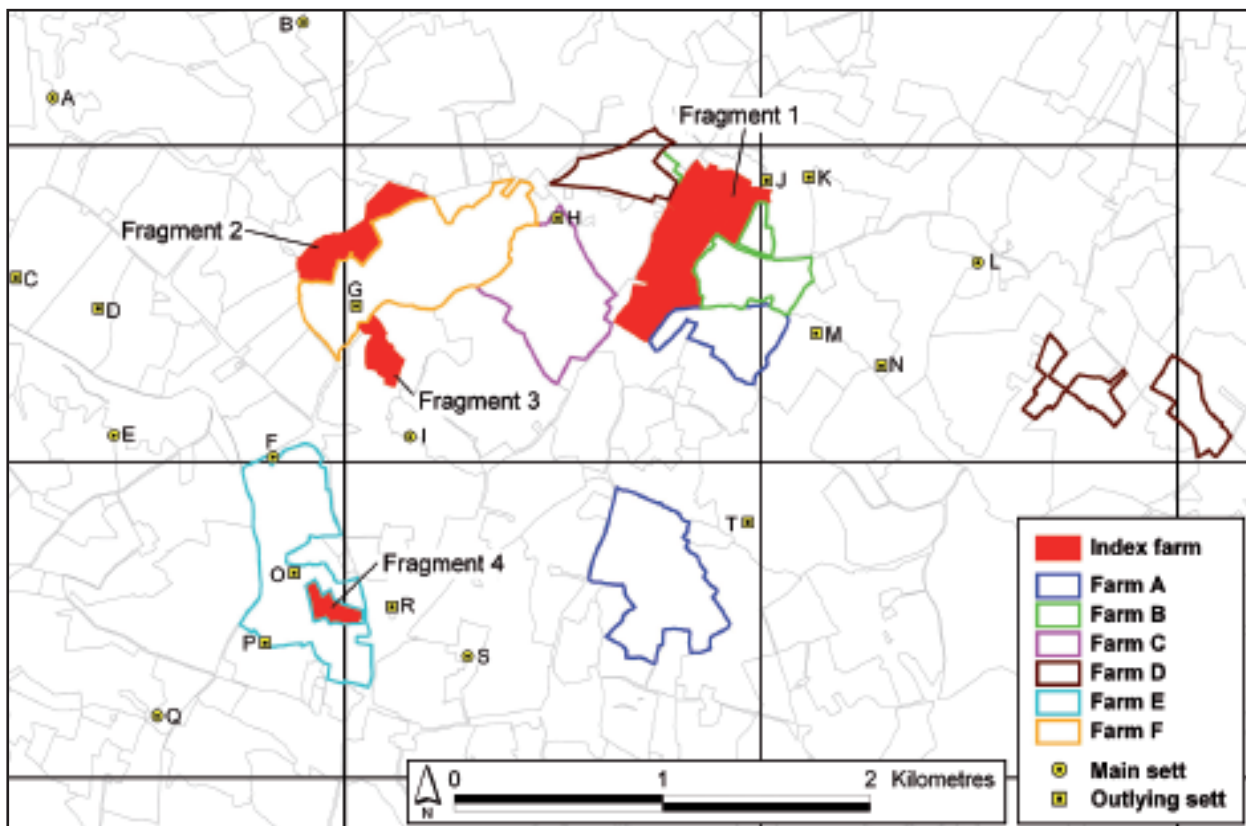
“Although factory surveillance is not the primary method for detecting *M. bovis*-infected animals in Irish herds, it plays an important role in the early detection of infected herds and in the detection of animals that are not reactive to the tuberculin test.”

AN OUTBREAK OF TUBERCULOSIS AFFECTING CATTLE AND PEOPLE ON AN IRISH DAIRY FARM IN 2005, FOLLOWING THE CONSUMPTION OF RAW MILK FROM A COW WITH TUBERCULOUS MASTITIS

Paul Doran (DAFF), Simon More (UCD CVERA), John Carson (Wexford General Hospital), Eamon Costello (DAFF)

Featured in the 'Selected reports' section

In Ireland, human infection with *M. bovis* is rare. Nonetheless, spillover of infection from cattle to people remains an ever-present possibility, given the current pool of infection in the Irish cattle population. This paper describes an outbreak of tuberculosis affecting cattle and people on a dairy farm in 2005, following the consumption of raw milk from a cow with tuberculous mastitis.



A map of the index farm, including fragments 1 (the home farm), 2 (yearling summer grazing), 3 (tillage only) and 4 (silage production, autumn grazing for calves). The location of six neighbouring farms (A to F) and local badger setts (differentiate by sett type) are also indicated

ADDITIONAL WORK

a. Genetics

GENETICS OF PREDISPOSITION TO TUBERCULOSIS IN IRISH DAIRY AND BEEF CATTLE

Máiréad Bermingham, Donagh Berry (Teagasc Moorepark), Margaret Good (DAFF), Simon More (UCD CVERA)

Little is known about the genetics of tuberculosis in cattle. 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 heritability) for susceptibility to tuberculosis, as well as possible genetic associations with other economically important traits. The interaction between gene expression and environment will also be evaluated.

INNATE GENE REPRESSION ASSOCIATED WITH *MYCOBACTERIUM BOVIS* INFECTION IN CATTLE: TOWARD A GENE SIGNATURE OF DISEASE

Meade K.G., Gormley, E., Doyle, M.B., Fitzsimons, T., O'Farrelly, C., Costello, E., Keane, J., Zhao, Y., MacHugh, D.E.

BMC Genomics 8 (2006), 400.

The advent of high-throughput functional genomics technologies has facilitated large-scale analyses of the immune response to this disease that may ultimately lead to novel diagnostics and therapeutic targets. Analysis of mRNA abundance in peripheral blood mononuclear cells (PBMC) from six *Mycobacterium bovis* infected cattle and six non-infected controls was performed. A targeted immunospecific bovine cDNA microarray with duplicated spot features representing 1,391 genes was used to test the hypothesis that a distinct gene expression profile may exist in *M. bovis* infected animals *in vivo*. These results suggest that large-scale expression profiling can identify gene signatures of disease in peripheral blood that can be used to classify animals on the basis of *in vivo* infection, in the absence of exogenous antigenic stimulation.

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“Gene signatures of disease can be used to classify animals on the basis of *M. bovis* infection, in the absence of exogenous antigenic stimulation.”

b. On-farm production

MODELLING THE EFFECT OF BOVINE TUBERCULOSIS ON MILK PRODUCTION

Fiona Boland, Gabrielle Kelly (UCD Statistics), Margaret Good (DAFF), Simon More (UCD CVERA)

There is little information on the effect of TB on milk production in dairy cattle. The purpose of this study was to develop statistical models to describe the relationship between TB infection and milk production in dairy cows.

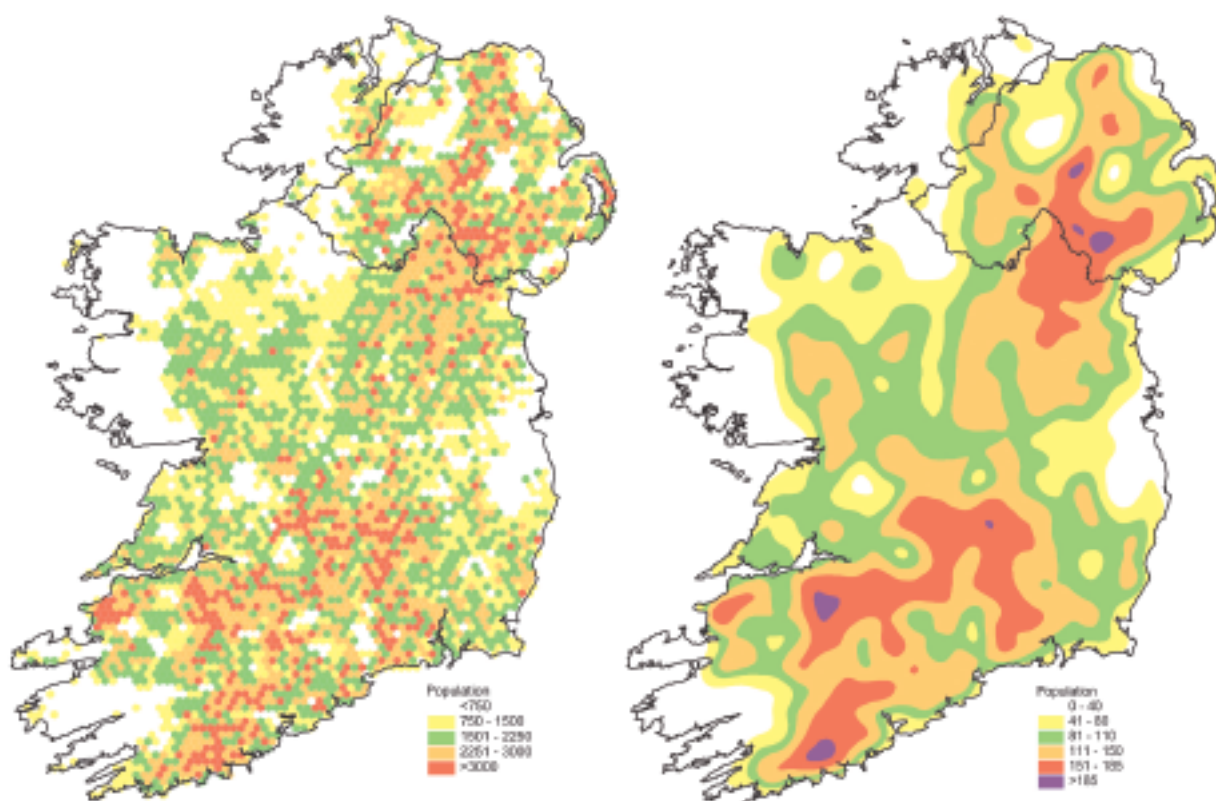
c. Contributing to North-South collaboration

AN ALL-ISLAND APPROACH TO MAPPING BOVINE TUBERCULOSIS IN IRELAND

Guy McGrath (UCD CVERA), Darrell Abernethy, Lesley Stringer (DARDNI, Belfast), Simon More (UCD CVERA)

Featured in the 'Selected reports' section

Bovine tuberculosis remains an important animal health issue throughout the island of Ireland. There have been similarities, but also differences, in eradication measures for this disease in Northern Ireland and the Republic of Ireland, which share a lengthy common border. The aim of this project is to use GIS to explore the spatial patterns of TB in the whole island over an 11-year period.



Representation of the TB-tested cattle population on the island of Ireland

THE DEFINITION OF AN EPIDEMIOLOGICAL UNIT IN THE CONTEXT OF DISEASE SURVEILLANCE AND CONTROL

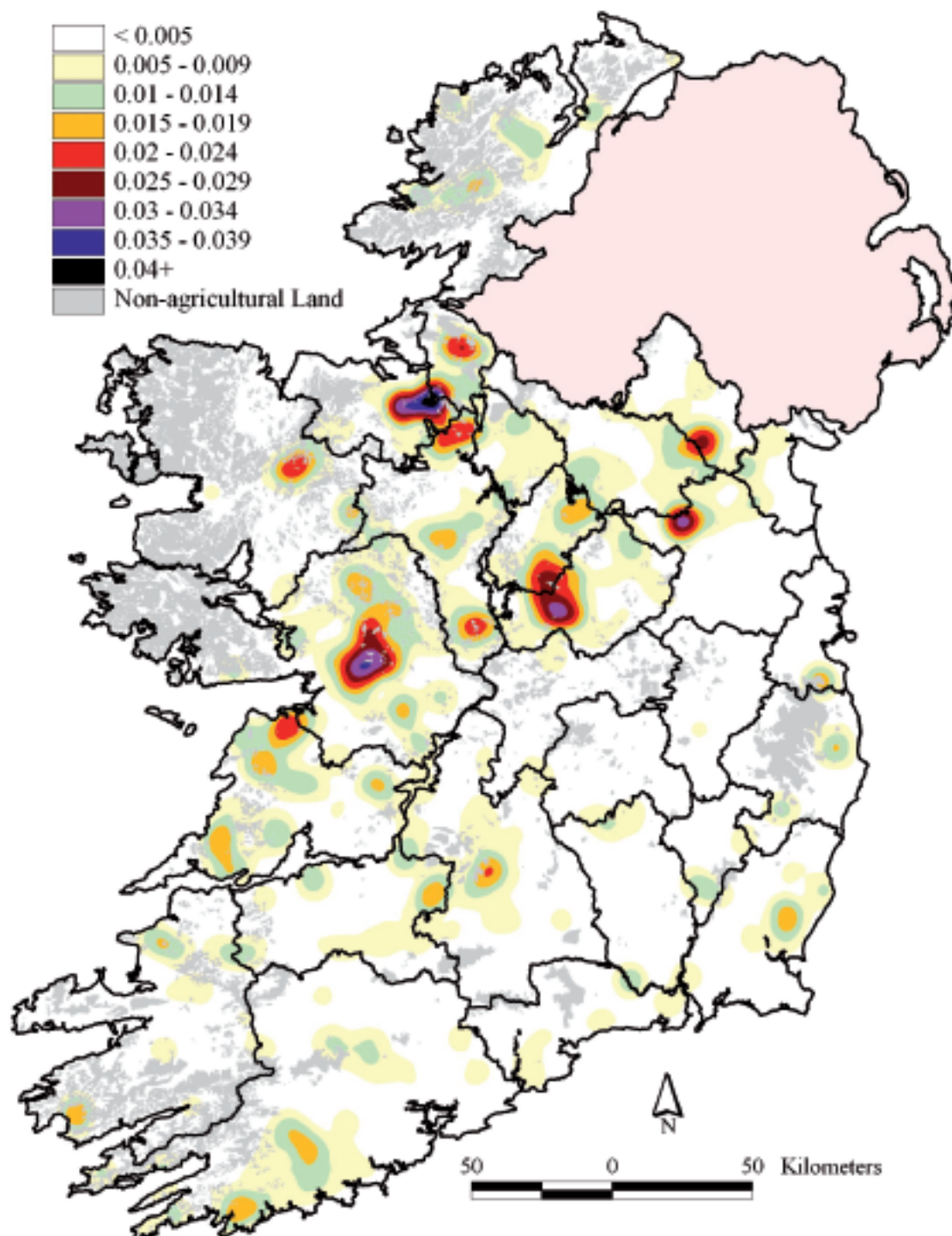
Darrell Abernethy, Fraser Menzies, Nigel Clark (DARDNI, Belfast), Simon More (UCD CVERA), Margaret Good (DAFF)

A North-South working group is seeking to address each of the following questions:

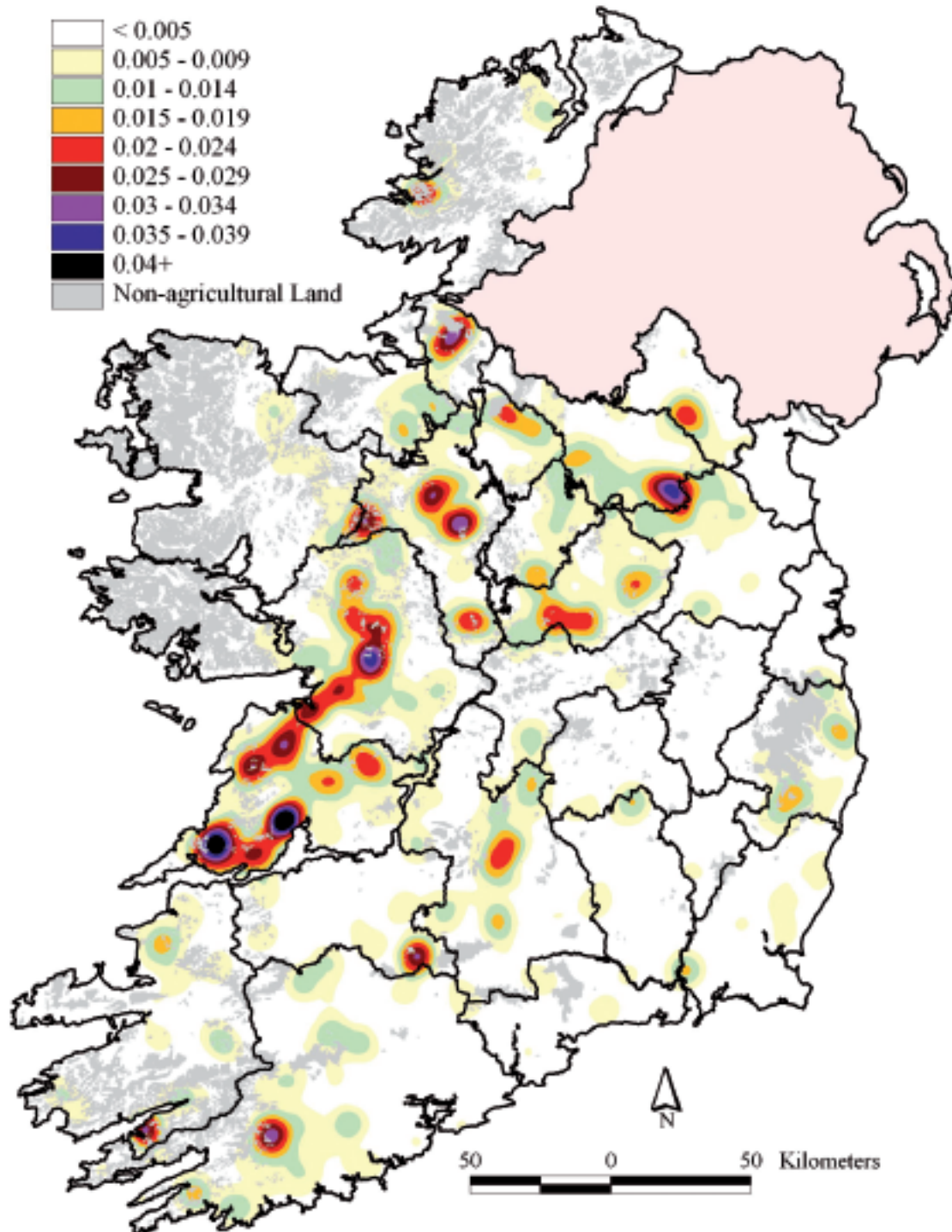
- Are 'herd' and 'epidemiological unit' synonymous?
- Do we agree with the interpretation of the EU concerning epidemiological units?
- Are Northern Ireland and the Republic of Ireland appropriately considering the concept of epidemiological units within the respective TB eradication programmes?

In other words, is herd fragmentation a constraint that has not been adequately addressed in the programmes?

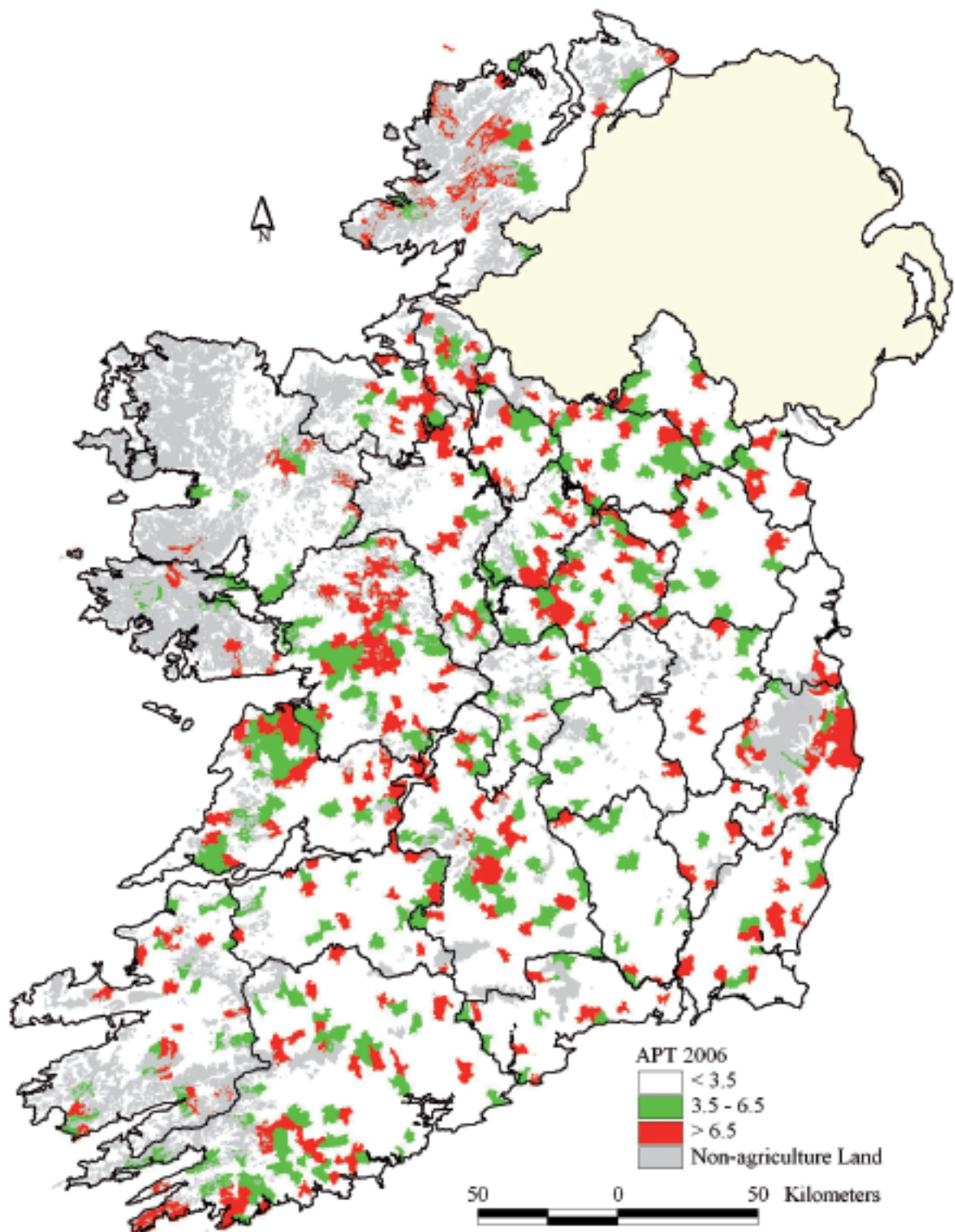
NATIONAL MAPS



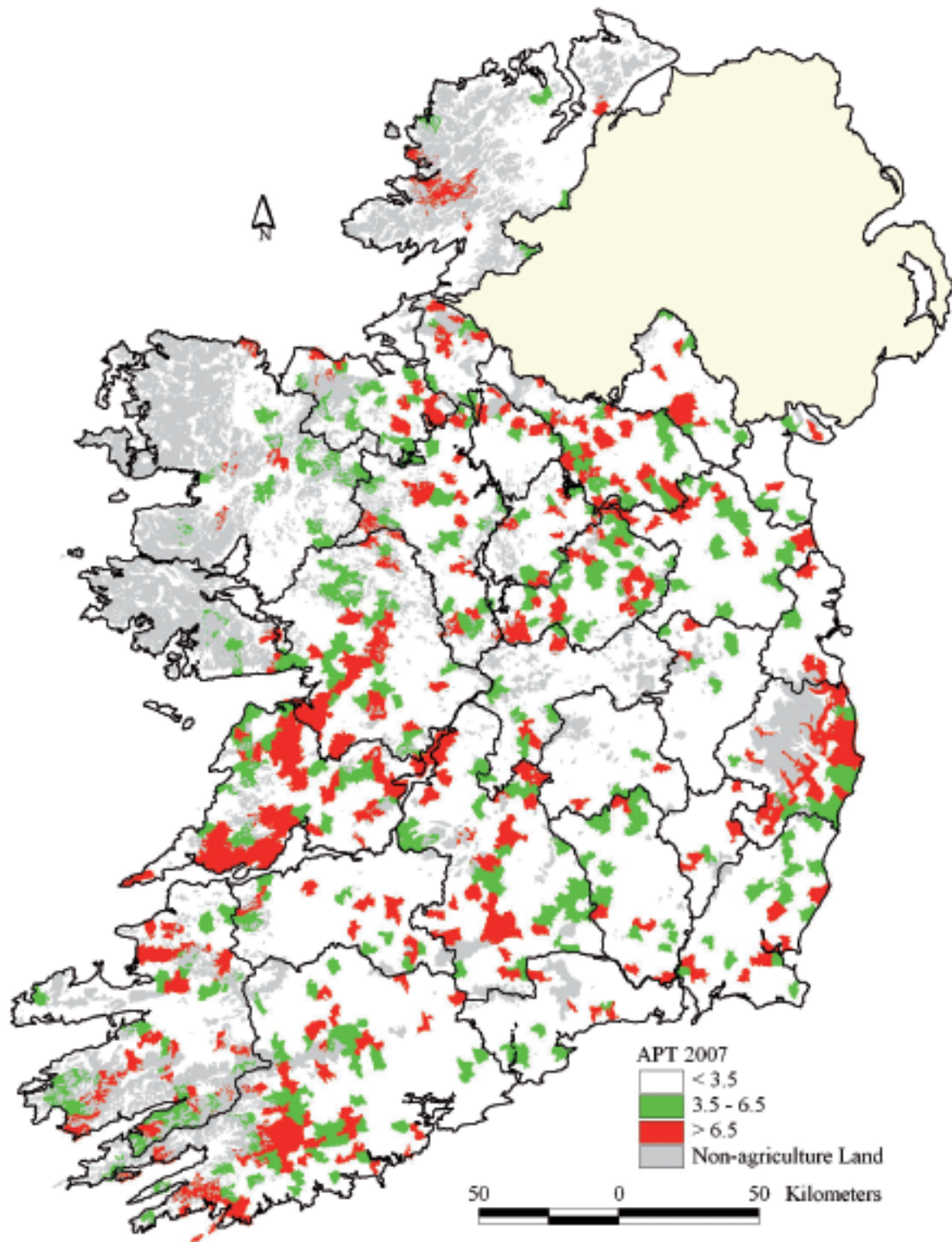
Density of TB incidence per square km during 2006 (kernel density with search radius at 10km)



Density of TB incidence per square km during 2007 (kernel density with search radius at 10km)



APT (reactors per 1000 tests) per district electoral division, 2006



APT (reactors per 1000 tests) per district electoral division, 2007

