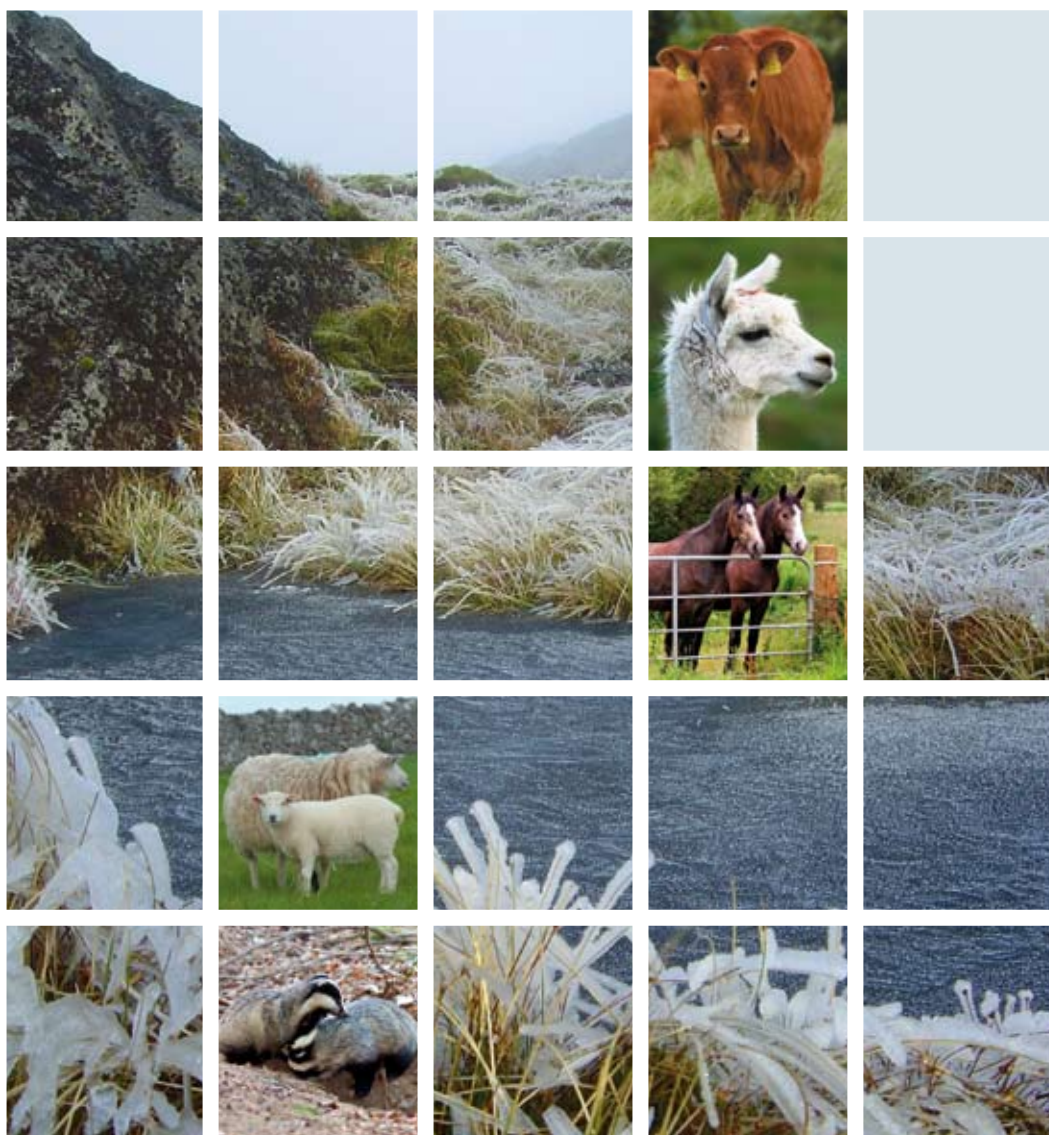| Title	The Centre for Veterinary Epidemiology and Risk Analysis, The TB Diagnostics and Immunology Research Centre, The Badger Vaccine Project. Biennial Report, 2008-09
Publication date	2010-05
Publication information	More, Simon John, and Daniel M. Collins, eds. The Centre for Veterinary Epidemiology and Risk Analysis, The TB Diagnostics and Immunology Research Centre, The Badger Vaccine Project. Biennial Report, 2008-09. University College Dublin. Centre for Veterinary Epidemiology and Risk Analysis, May, 2010.
Series	Biennial Reports
Publisher	University College Dublin. Centre for Veterinary Epidemiology and Risk Analysis
Item record/more information	http://hdl.handle.net/10197/8908

The Centre for Veterinary Epidemiology and Risk Analysis
The TB Diagnostics and Immunology Research Centre
The Badger Vaccine Project

Biennial Report, 2008-09

UCD DUBLIN

Department of **Agriculture, Fisheries and Food**
An Roinn **Talmhaíochta, Iascaigh agus Bia**

The Centre for Veterinary Epidemiology and Risk Analysis

The TB Diagnostics and Immunology Research Centre

The Badger Vaccine Project

Biennial Report, 2008-09
S.J. More and D.M. Collins (editors)

Preface

The Department of Agriculture, Fisheries and Food (DAFF) provides ongoing financial support to three research units within the UCD School of Agriculture, Food Science and Veterinary Medicine at University College Dublin:

- The Centre for Veterinary Epidemiology and Risk Analysis (CVERA);
- The TB Diagnostics and Immunology Research Centre; and
- The Badger Vaccine Project.

These units each work to support DAFF policy, inspectorate and laboratory staff in the area of animal health. The TB Diagnostics and Immunology Research Centre and the Badger Vaccine Project focus on bovine tuberculosis research. CVERA is a national resource centre, providing policy advice and conducting epidemiological research on a wide range of animal health issues. In addition, CVERA provides general support to government, industry and the veterinary profession (pre- and post-graduation) on these and other animal health issues.

This report documents work conducted by, or in association with, these three UCD-based research units during 2008 and 2009.

Simon J. More
Eamonn Gormley
Leigh Corner

Veterinary Sciences Centre
UCD School of Agriculture, Food Science and Veterinary Medicine
University College Dublin
Belfield, Dublin 4, Ireland

The UCD Veterinary Sciences Centre. Photograph by D.M. Collins.

Acknowledgements

The Centre for Veterinary Epidemiology and Risk Analysis

CVERA works closely with colleagues from a wide range of organisations, as listed below, both in Ireland and internationally. Their input is gratefully acknowledged.

Further information

In this report, projects are either:

- Complete, which includes those projects where relevant peer-reviewed papers, or equivalent, have been published, or
- Current, which includes the balance covering the spectrum from conceptual through to final write-up.

Manuscript preparation is conducted in accordance with Uniform Requirements for Manuscripts Submitted to Biomedical Journals of the International Committee of Medical Journal Editors (previously the Vancouver Group). For further information, see **www.icmje.org.** Guidelines for the transparent reporting of specific study types (for example, the CONSORT statement for transparent reporting of trials, **www.consort-statement.org**) are followed.

An up-to-date list of all peer-review papers produced by, or in association with, the Centre for Veterinary Epidemiology and Risk Analysis, the TB Diagnostics and Immunology Research Centre and the Badger Vaccine Project is available at **www.ucd.ie/cvera**.

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Gabrielle Kelly and David Williams, UCD School of Mathematical Sciences, University College Dublin, Ireland

Paddy Sleeman, Department of Zoology, Ecology and Plant Science, University College Cork

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Francisco Olea-Popelka, Department of Clinical Sciences, Colorado State University, United States of America

Mart de Jong and Klaas Frankena, University of Wageningen, The Netherlands

The TB Diagnostics and Immunology Research Centre

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Overview

The Centre for Veterinary Epidemiology and Risk Analysis

The Centre for Veterinary Epidemiology and Risk Analysis (CVERA) is the national resource centre for veterinary epidemiology in Ireland, located within the UCD School of Agriculture, Food Science and Veterinary Medicine at University College Dublin. The Centre was initially established as the Tuberculosis Investigation Unit, but in recent years has broadened its remit to cover a wide range of international, national and local animal health matters, including:

- Epidemiological support for the control and eradication of regulatory animal diseases, which includes national programmes for bovine tuberculosis, bovine brucellosis and bovine spongiform encephalopathy;

- Epidemiological support for a broad range of other animal health and welfare issues relating to emergency animal disease preparedness and response (for example, avian influenza, bluetongue and equine infectious anaemia), on-farm investigations, welfare of farmed livestock and horses, health of companion animals and farmed fish, and international collaboration; and

- Work in support of Animal Health Ireland, which is seeking to provide a proactive, coordinated and industry-led approach in Ireland to non-regulatory animal health concerns (such as mastitis, fertility and infectious bovine rhinotracheitis).

CVERA staff work closely with national policy-makers, both in government and industry. In collaboration with staff from the UCD School of Agriculture, Food Science and Veterinary Medicine, CVERA staff also contribute to on-farm animal health investigations throughout Ireland. A broad range of expertise is represented within the Centre, including agriculture and animal sciences, database development and management, geographic information systems, statistics, veterinary medicine and epidemiology. The Centre is staffed by employees of University College Dublin and of the Department of Agriculture, Fisheries and Food (DAFF).

The TB Diagnostics and Immunology Research Centre

The gamma-interferon (IFN-γ) assay (Bovigam) is used as a tool to assist in the eradication of bovine tuberculosis from the national cattle herd. All of the testing is carried out in the laboratory based at UCD. In the period 2008-2009, over 28,000 blood samples were submitted to the laboratory for testing, including 1,300 goats. The majority of samples originated from bovine reactor re-test herds where the test was used to identify infected animals that were missed by the skin test. Other strategic uses of the test were targeted at inconclusive reactor re-tests. The apparent sensitivity and specificity of the IFN-γ test was re-assessed in a large-scale study involving several thousand cattle. This was part of a larger project to compare the performance of the skin test, IFN-γ test and a new serology test developed by Enfer Group. The results of this study have helped our understanding of how the test performs in the cohorts tested. In 2010 we await the delivery of the next generation Bovigam test being developed by Prionics Ltd. The test promises improved specificity without loss of sensitivity. The laboratory at UCD will be involved in evaluating this test under Irish conditions.

The Badger Vaccine Project

The Badger Vaccine Project is a comprehensive programme of research that seeks to develop a vaccine to control tuber-culosis in badgers and to break the link of infection to cattle. We have demonstrated that vaccination of badgers with BCG by a number of routes, including oral delivery, generates high levels of protective immunity against challenge with *M. bovis*. The key to the success of the vaccine lies in the encapsulation of the vaccine in a specific lipid formulation that protects it from degradation as it passes through the stomach. The encapsulation technology designed for this purpose has been developed by collaborators in Otago, New Zealand. We are continuing to carry out studies with captive popu-lation of badgers to refine the vaccine and address issues relating to the eventual registration of the vaccine as a veterinary medicine. We are also developing and evaluating diagnostic tests with colleagues at VLA (Weybridge UK). The results of our studies to date have increased our understanding of the progression of the disease following infection and have improved our ability to accurately diagnose *M. bovis* infection in badgers. A field trial commenced in 2009 to test the efficacy of the oral BCG vaccine with a large number of badgers over a wide geographic area in Co. Kilkenny. Success in the field trial will lead to implementation of a vaccination strategy into the national control programme.

Bovine tuberculosis

Improving surveillance

Field-based surveillance

Multiplex immunoassay for serological diagnosis of *Mycobacterium bovis* infection in cattle

Whelan, C.[1], Shuralev, E.[1], O'Keeffe, G.[1], Hyland, P.[1], Kwok, H.F.[2], Snoddy, P.[2], O'Brien, A.[1], Connolly, M.[1], Quinn, P.[1], Groll, M.[3], Watterson, T.[3], Call, S.[3], Kenny, K.[4], Duignan, A.[5, 8], Hamilton, M.J.[6], Buddle, B.M.[7], Johnston, J.A.[2], Davis, W.C.[6], Olwill, S.A.[2], Clarke, J.[1]

[1] Enfer Scientific, [2] Fusion Antibodies Ltd., Northern Ireland, [3] Quansys Biosciences, Utah, USA, [4] Central Veterinary Research Laboratory, Department of Agriculture, Fisheries and Food, [5] Department of Agriculture, Fisheries and Food, [6] Washington State University, USA, [7] AgResearch, Hopkirk Research Institute, Palmerston North, New Zealand, [8] UCD CVERA

Efforts to develop a better diagnostic assay for bovine tuberculosis have shown that the sensitivity and specificity of an assay can be improved by the use of two or more antigens. As reported here, we developed a multiplex chemiluminescent immunoassay that can simultaneously detect antibody activity to 25 antigens in a single well in a 96-well plate array format. The chemiluminescent signal is captured with a digital imaging system and analyzed with a macro program that tracks each serum for its pattern of antibody activity for *Mycobacterium bovis* antigens. The comparison of sera from 522 infected and 1,489 uninfected animals showed that a sensitivity of 93.1% and a specificity of 98.4% can be achieved with a combination of antigens. The assay system is rapid and can be automated for use in a centralized laboratory.

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

Clegg, T.A.[1], Duignan, A.[1, 2], Whelan, C.[3], Gormley, E.[4], Good, M.[2], Clarke, J.[3], Toft, N.[5], More, S.J.[1]

[1] UCD CVERA, [2] Department of Agriculture, Fisheries and Food, [3] Enfer Scientific, [4] UCD TB Diagnostics and Immunology Research Centre, [5] University of Copenhagen, Copenhagen, Denmark

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

Enferplex-TB[level-1] (95% PCI: 64.8-71.9%) was superior to the SICTT[standard], and specificity of the Enferplex-TB[level-5] was superior to γ IFN (95% PCI: 99.6-100.0%). These results provide robust measures of sensitivity and specificity under field conditions in Ireland and suggest that the Enferplex-TB test has the potential to improve on current diagnostics for TB infection in cattle.

Further test characterisation (comparison of herd-level classification using SICTT and Enfer)

Good, M.[1], Clegg, T.A.[2]

[1] Department of Agriculture, Fisheries and Food, [2] UCD CVERA

For the purpose of this study DVOs submitted blood samples for analysis from herds undergoing high risk or consequential test type tests (i.e. not round tests) and which represent the whole of Ireland. Samples were also 'captured' from the Brucellosis Laboratory for herds undergoing routine round tests. In total some 100,000 samples were analysed by Enfer Scientific in the latter months of 2009. The principal research questions to be addressed by this study are as follows:

- Is there agreement in the herd-level interpretation of the SICTT and Enferplex tests?
- If not, what is the confirmation rate among SICTT herds where no animals were Enferplex positive?
- What is the future outcome of herds/animals that were Enferplex positive but SICTT negative?

Meta-analysis of diagnostic tests and modelling to identify appropriate testing strategies to reduce *Mycobacterium bovis* infection in cattle

Downs, S.[1], Parry, J.[1], Abernethy, D.[2], Broughan, J.[1], Cameron, A.[3], Clifton-Hadley, R.[1], Cook, A.[1], Cox, D.[4], de la Rua-Domenech, R.[5], Goodchild, T.[1], Greiner, M.[6], Gunn, J.[1], More, S.J.[7], Nunez-Garcia, J.[1], Sharp, M.[1], Rhodes, S.[1], Rolfe, S.[8], Upton, P.[1], Vordermeier, M.[1], Watson, E.[1], Welsh, M.[9], Whelan, A.[1], Woolliams, J.[10]

[1] Veterinary Laboratories Agency, Weybridge, Surrey, England, [2] Department of Agriculture and Rural Development, Belfast, Northern Ireland, [3] AusVet Animal Health Services, Cuiseaux, France, [4] Nuffield College, University of Oxford, Oxford, England, [5] Department of Environment, Food and Rural Affairs, United Kingdom, [6] Federal Institute for Risk Assessment (BfR), Berlin, Germany, [7] UCD CVERA, [8] Welsh Assembly Government, Cardiff, Wales, [9] Agri-Food & Biosciences Institute (AFBI), Belfast, Northern Ireland, [10] Roslin Institute of the University of Edinburgh, Scotland

In this collaborative project, a comprehensive systematic literature review has been undertaken of studies that have measured the performance of tests for diagnosing bovine tuberculosis in cattle. Estimates of test performance and the characteristics of the studies and populations in which the studies were conducted have been extracted from the literature, following a standardised procedure. The results will be pooled and a statistical meta-analysis conducted to obtain estimates with distributions for the sensitivities and specificities of the tests. The second part of the project is the development of a model to explore the improved use of the tests in bovine tuberculosis surveillance and control. The model estimates the probability that a herd with specified characteristics and surveillance history is free from infection. The model will be used to evaluate a range of potential future surveillance options, based on sensitivity, specificity, the probability of introduction of infection into a herd and the time required to achieve *M. bovis* infection free status.

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

Clegg, T.A.[1], Good, M.[2], Duignan, A.[1, 2], More, S.J.[1]

[1] UCD CVERA, [2] Department of Agriculture, Fisheries and Food

In Ireland, cattle are tested annually, using the single intradermal comparative tuberculin test (SICTT). Quality control (QC) is an important part of the national programme, and new methods are progressively being introduced. Field surveillance using the SICTT is potentially problematic, noting its reliance on a range of factors, including the skills and experience of the tester. The objective of the current study was to quantify the relative effectiveness of, and reporting accuracy among, testers during field surveillance for tuberculosis in Ireland. All testers who carried out at least one annual herd test in 2006 were included in the study. Relative testing effectiveness was assessed by comparing the number of observed and expected herd restrictions per tester. The latter was predicted, using a logistic regression model and testers were ranked, based on OEDIFF. Reporting accuracy was based on the bovine measurements in SICTT negative animals. In total, 983 testers were enrolled in the study, including data from 92,402 herds, including 1,839 (1.99%) with at least one reactor at the eligible test. OEDIFF varied from -7.5 to 18.2; for 43% of testers, OEDIFF was between -1.0 and 1.0. There was evidence of reduced testing effectiveness among a small number of testers. Reporting accuracy was low suggesting under-reporting of bovine measurements. This study provides objective data on the relative performance of testers during field surveillance in Ireland. Output from these models will be used as part of ongoing QC activities in the national programme.

A review of quality control in the national bovine tuberculosis eradication programme in Ireland

Duignan, A.[1, 2], Good, M.[1], More, S.J.[2]

[1] Department of Agriculture, Fisheries and Food, [2] UCD CVERA

Quality control (QC) is a recognised process in the delivery of quality products or services. The application of QC in veterinary laboratories has been described in some detail; as yet, however, little has been written about QC in other aspects of national animal disease control programmes. This paper presents a review of QC in the national bovine tuberculosis (BTB) eradication programme in Ireland, with particular emphasis on QC processes for Private Veterinary Practitioners (PVPs) who carry out much of the field surveillance using the Single Intradermal Comparative Tuberculin Test (SICTT). The Irish BTB eradication programme operates under national legislation and at the same time fulfils the requirements of the EU trade Directive 64/432. The programme includes annual SICTT screening of all herds, prompt removal of test reactors and further consequential retesting of herds. Continuous evaluation of all relevant activities is essential to deliver an effective national programme and to reassure all stakeholders including taxpayers, producers and export markets that the highest possible standards are attained. A broad range of programme elements subjected to QC, are described, including personnel, training, equipment, tuberculins and laboratory. Each element is relevant to activities within both field and abattoir surveillance. Particular attention is paid to field surveillance (specifically, PVP performance), following the recent introduction of the national Animal Health Computer System (AHCS). Specialist performance reports have been produced, focusing on measures relating to administrative functions and disease control. The measures were chosen as objective and measurable. They are perceived as the best available and the process is constantly evolving as part of a work in progress. While attention has been directed to the issue of standards of SICTT delivery by PVPs, little has been documented in published literature on the subject of Quality Control and Quality Assurance in disease eradication programmes. This paper seeks to fill that gap.

Factory surveillance

Quantification of the relative efficiency of factory surveillance in the disclosure of tuberculosis lesions in attested Irish cattle, 2005-2007

Olea-Popelka, F.J.[1], Freeman, Z.[1], White, P.[2, 3], Costello, E.[4], O'Keeffe, J.[2, 3], Frankena, K.[5], Martin, S.W.[6], More, S.J.[3]

[1] Department of Clinical Sciences, College of Veterinary Medicine and Biomedical Sciences, Colorado State University, Colorado, USA, [2] Department of Agriculture, Fisheries and Food, [3] UCD CVERA, [4] Central Veterinary Research Laboratory, Department of Agriculture, Fisheries and Food, [5] Wageningen Institute of Animal Sciences, Wageningen University, The Netherlands, [6] Department of Population Medicine, Ontario Veterinary College, University of Guelph, Ontario, Canada

In Ireland, as part of the ongoing Bovine Tuberculosis (BTB) eradication scheme, every animal is examined at slaughter for its fitness for human consumption. Between 1982 and 1999, the annual risk of BTB lesion detection among animals from herds considered to be free of BTB was 0.12% (on average 1,638 animals per year) (Byrne, 2000). The aim of this study was to determine the relative efficiency of factories in detecting, submitting and subsequently confirming BTB lesion as caused by *Mycobacterium bovis* among attested cattle (considered free of BTB) between years 2005-2007. Factories were ranked according their submission and confirmation risk, adjusting for the risk profile of the animals slaughtered, including potential confounding factors such as age, gender, whether they were homebred or purchased, the test history of their herd, the prevalence of BTB in the herd geographical area and the season of slaughter. Approximately, 4.9 million cattle were slaughtered in 37 Irish export-licensed factories in these three years. Complete data were available for 3,344,057 animals from 89,870 attested herds in 2830 District Electoral Divisions. Samples from 11,530 attested animals with suspected BTB lesions were submitted for laboratory examination, 7,900 (68.5%) were positive, 3,238 were negative and 392 were inconclusive. Samples from 8,178 animals with complete information regarding potential confounding factors with suspected BTB lesions were submitted for laboratory examination, and from these 5,456 (66.7%) were positive, 2453 were negative and 269 were inconclusive. The average unadjusted submission risk for all the factories was 25 per 10,000, ranging from 0 to 52 per 10,000. The unadjusted confirmation risk varied between 30.3% and 91.3%.
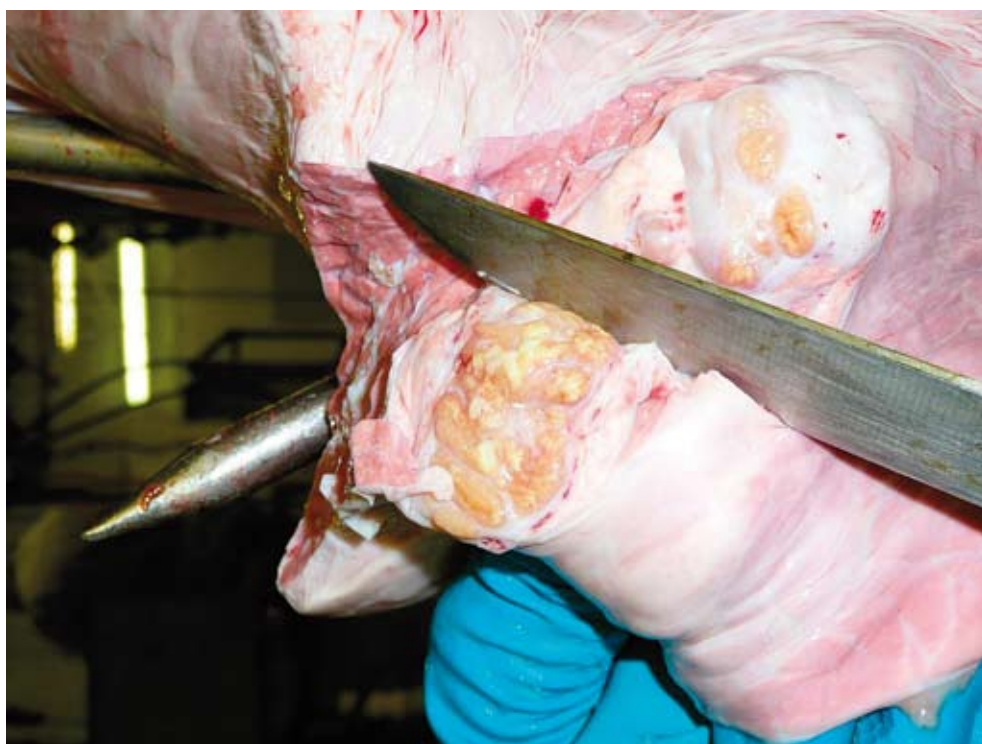
Incised lung during factory surveillance. Photograph by A. Duignan.

Supporting work

Antigen stimulation of peripheral blood mononuclear cells from *Mycobacterium bovis* infected cattle yields evidence for a novel gene expression program

Meade, K.G.[1], Gormley, E.[2], O'Farrelly, C.[1], Park, S.D.[3], Costello, E.[4], Keane, J.[5], Zhao, Y.[6], MacHugh, D.E.[3, 7]

[1] School of Biochemistry and Immunology, Trinity College Dublin, [2] UCD TB Diagnostics and Immunology Research Centre, [3] UCD School of Agriculture, Food Science and Veterinary Medicine, [4] Central Veterinary Research Laboratory, Department of Agriculture, Fisheries and Food, [5] School of Medicine, Trinity College Dublin, [6] Computational and Systems Biology Group, Biometric Research Branch, National Cancer Institute, Maryland, USA, [7] UCD Conway Institute of Biomolecular and Biomedical Research

Bovine tuberculosis (BTB) caused by *Mycobacterium bovis* continues to cause substantial losses to global agriculture and has significant repercussions for human health. The advent of high throughput genomics has facilitated large scale gene expression analyses that present a novel opportunity for revealing the molecular mechanisms underlying mycobacterial infection. Using this approach, we have previously shown that innate immune genes in peripheral blood mononuclear cells (PBMC) from BTB-infected animals are repressed in vivo in the absence of exogenous antigen stimulation. In the present study, we hypothesized that the PBMC from BTB-infected cattle would display a distinct gene expression program resulting from exposure to *M. bovis*. A functional genomics approach was used to examine the immune response of BTB-infected (n = 6) and healthy control (n = 6) cattle to stimulation with bovine tuberculin (purified protein derivative - PPD-b) in vitro. PBMC were harvested before, and at 3 h and 12 h post in vitro stimulation with bovine tuberculin. Gene expression changes were catalogued within each group using a reference hybridization design and a targeted immunospecific cDNA microarray platform (BOTL-5) with 4,800 spot features representing 1,391 genes. 250 gene spot features were significantly differentially expressed in BTB-infected animals at 3 h post-stimulation contrasting with only 88 gene spot features in the non-infected control animals ($P < $ or $= 0.05$). At 12 h post-stimulation, 56 and 80 gene spot features were differentially expressed in both groups respectively. The results provided evidence of a proinflammatory gene expression profile in PBMC from BTB-infected animals in response to antigen stimulation. Furthermore, a common panel of eighteen genes, including transcription factors were significantly expressed in opposite directions in both groups. Real-time quantitative reverse transcription PCR (qRT-PCR) demonstrated that many innate immune genes, including components of the TLR pathway and cytokines were differentially expressed in BTB-infected (n = 8) versus control animals (n = 8) after stimulation with bovine tuberculin. The PBMC from BTB-infected animals exhibit different transcriptional profiles compared with PBMC from healthy control animals in response to *M. bovis* antigen stimulation, providing evidence of a novel gene expression program due to *M. bovis* exposure.

Gene expression profiling of the host response to *Mycobacterium bovis* infection in cattle

MacHugh, D.E.[1, 2], Gormley, E.[3], Park, S.D.[1], Browne, J.A.[1], Taraktsoglou, M.[1], O'Farrelly, C.[4], Meade, K.G.[4]

[1] UCD School of Agriculture, Food Science and Veterinary Medicine, [2] UCD Conway Institute of Biomolecular and Biomedical Research, [3] UCD TB Diagnostics and Immunology Research Centre, [4] Comparative Immunology Group, School of Biochemistry and Immunology, Trinity College Dublin

Bovine tuberculosis (BTB), caused by *Mycobacterium bovis*, continues to pose a threat to livestock worldwide and, as a zoonotic infection, also has serious implications for human health. The implementation of comprehensive surveillance programmes to detect BTB has been successful in reducing the incidence of infection in many countries, yet BTB has remained recalcitrant to eradication in several EU states, particularly in Ireland and the UK. There are well-recognized limitations in the use of the current diagnostics to detect all infected animals and this has led to renewed efforts to uncover novel diagnostic biomarkers that may serve to enhance the performance of the tests. Studies of single immuno-logical parameters have so far been unable to unlock the complexities of the immune response to mycobacterial infection. However, the development of high-throughput methods including pan-genomic gene expression technologies such as DNA microarrays has facilitated the simultaneous identification and analysis of thousands of genes and their interac-tions during the immune response. In addition, the application of these new genomic technologies to BTB has identified pathogen-associated immune response signatures of host infection. The objective of these investigations is to understand the changing profile of immune responses throughout the course of infection and to identify biomarkers for sensitive diagnosis, particularly during the early stages of infection. Transcriptional profiling via microarray and more recently via next-generation sequencing technologies may lead to the development of specific and sensitive diagnostics for *M. bovis* infection and will enhance the prospect of eradication of tuberculosis from cattle populations.

Photograph courtesy of Bord Bia.

Improving management of high risk herds

TB epidemiology

Risk of bovine tuberculosis (BTB) for cattle sold out from attested herds during year 2005 in Ireland

Berrian, A.M.[1], O'Keeffe, J.[2, 3], White, P.[2, 3], Olea-Popelka, F.[4]

[1] Colorado School of Public Health, Colorado, USA, [2] Department of Agriculture, Fisheries and Food, [3] UCD CVERA, [4] Department of Clinical Sciences, College of Veterinary Medicine and Biomedical Sciences, Colorado State University, Colorado, USA

Bovine tuberculosis (BTB) is an infectious contagious disease caused by a bacterium *Mycobacterium bovis*. The significance of BTB lies in trade implications and its zoonotic potential. Although industrialized countries have significantly reduced the prevalence of *M. bovis* infection in both humans and animals, BTB persists in many developed countries, including the Republic of Ireland. A retrospective cohort study was conducted to determine the risk of BTB among animals sold out from attested (BTB "free") herds during the year 2005. Herds from which the animals were sold out were classified as "exposed" and "non-exposed" to BTB according to their BTB history during the year 2005. The study sample was comprised of 338,960 animals, from which 124,360 were sold out from "exposed" herds and 214,600 animals were sold out from "non-exposed" herds. After the selling date during the year 2005, animals were followed until the end of the year 2007. All animals reacting positive to the Single Intradermal Comparative Tuberculin Test (SICTT) as well as all animals that disclosed a BTB lesion at slaughter that subsequently was confirmed as *M. bovis* positive at the laboratory were classified as BTB positive. The overall risk of BTB during the study period after the animals were sold out was 0.69%. Logistic regression analysis indicated that the odds of being found BTB positive was greater for animals sold out from "exposed" herds compared to animals sold out from "non-exposed" herds (OR=1.94, 95% CI = 1.79-2.11, p = < 0.0001). Other risk factors, such as age and gender, were also significantly associated with the risk of BTB at the animal level in Ireland. Due to the contagious nature of BTB, the identification and quantification of risk factors associated with the development of the disease is essential to its control at the animal level. These are preliminary findings from a study to be completed during the year 2010. This study will provide quantitative epidemiological information at the animal level that can be used to further assess Ireland's progress towards BTB eradication.

Tuberculosis in a dairy farm in Ireland: a case study

Meaney, L.[1], Kenny, K.[2], Johnson, A.[3], Good, M.[1]

[1] Department of Agriculture, Fisheries and Food, [2] Central Veterinary Research Laboratory, Department of Agriculture, Fisheries and Food,

[3] Limerick Regional Veterinary Laboratory, Department of Agriculture, Fisheries and Food

In a number of countries, bovine tuberculosis (BTB; due to infection with *Mycobacterium bovis*) is a significant animal health problem. In Ireland, BTB is a problem in the national cattle herd, and is endemic in badgers *(Meles meles)*. However, few case reports from Ireland are available. The aim of this paper is to describe an outbreak of bovine tuberculosis in a dairy herd on a farm in Ireland, where 3% of the herd was SICTT-positive at initial detection but where infection was eventually evident in all groups within the herd. The epidemiological investigation was conducted in an effort to determine the origin of the outbreak and within-herd spread, including consideration of the possibility of spread to and from people on the holding.

How important is introduced infection?

Potential infection-control benefit for Ireland from pre-movement testing of cattle for tuberculosis

Clegg, T.A.[1], More, S.J.[1], Higgins, I.[1], Good, M.[2], Blake, M.[2], Williams, D.H.[3]

[1] UCD CVERA, [2] Department of Agriculture, Fisheries and Food, [3] UCD School of Mathematical Sciences

Pre-movement testing for bovine tuberculosis (BTB) was compulsory in Ireland until 1996. We determined the proportion of herd restrictions (losing BTB-free status) attributable to the recent introduction of an infected bovid; described events between restoration of BTB-free status (de-restriction) and the next herd-level test for BTB; estimated the proportion of undetected infected cattle present at de-restriction; identified high-risk movements between herds (movements most likely to involve infected cattle); and determined the potential yield of infected cattle discovered (or herds that would not lose their BTB-free status) by pre-movement testing, relative to the numbers of cattle and herds tested. We used national data for all 6252 herds with a new BTB restriction in the 12 months from 1 April 2003 and 3947 herds declared BTB free in the 12 months from 1 October 2001. We identified higher-risk animals from our logistic generalized estimating-equation models. We attributed 6–7% of current herd restrictions to the recent introduction of an infected animal. There were considerable changes to herd structure between de-restriction and the next full-herd test, and infection was detected in 10% of herds at the first assessment (full-herd test or abattoir surveillance) following de-restriction. Following movement from a de-restricted herd, the odds of an animal being positive at the next test increased with increasing time in the source herd prior to movement, increasing time between de-restriction and the next full-herd test and increasing severity of the source herd restriction. The odds decreased with increasing size of the source herd. We estimated that 15.9 destination-herd restrictions per year could be prevented for every 10,000 cattle tested pre-movement and that 3.3 destination-herd restrictions per year could be prevented for every 100 source herds tested pre-movement. The yield per pre-movement test can be increased by focusing on high-risk movements; however, this would result in a substantial decrease in the total number of potential restrictions identified.

Predictors of the first between-herd animal movement for cattle born in 2002 in Ireland - the implications for control/eradication of bovine tuberculosis

White, P.[1,2], Frankena, K.[3], de Jong, M.[3], O'Keeffe, J.[1,2], Martin, W.[4]

[1] Department of Agriculture, Fisheries and Food, [2] UCD CVERA, [3] Wageningen Institute of Animal Sciences, Wageningen University, The Netherlands,

[4] Department of Population Medicine, Ontario Veterinary College, University of Guelph, Ontario, Canada

The movement of cattle between herds may constitute a risk for the transmission of bovine tuberculosis (BTB) and other disease transmission. The objectives of this study were to identify and quantify the risk factors associated with the first between-herd movement of animals (denoted as risk move) as an aide to control BTB in Ireland. A random sample of 1 percent of Irish calf births registered for 2002 (20,182 animals) was selected. Descriptive and survival analysis on movement over the period 2002-2005 was performed. Over the 4-year study period, 12,119 (60%) of animals experienced a risk move. Among those that moved, 57% did so within the first 12 months of age. For animals in dairy herds, an early peak in risk move events was observed within the first 12 weeks of age; whereas in animals from suckler herds, a later risk move peak was observed between 21 and 36 weeks of age. The survival models identified a number of risk factors:

two that appeared most important in predicting a risk move were gender and enterprise type. Males had a hazard ratio of 2.6 times that of females. The hazards for enterprise type, varied over time, thus a time-varying covariate (ent_type x ln(time)) was included in the Cox model. At 7 days of age, females in suckler herds were at 0.14 times the hazard of females in dairy herds for risk move, and over time, the hazards converged, equalised by day 140, and then diverged, so that by 4 years of age, females in suckler herds were at 4.64 times the hazard of females in dairy herds. Herds with a history of selling animals in previous years maintained that record during the study period with increased hazard of risk move. Enterprise type interacted with gender so that relative to females, males from dairy herds were at greater hazard of risk move than males from suckler herds. Hazard of risk move was also a function of ln(herd area), so that each doubling of farm area was accompanied by a 30.6% decrease in the hazards. When the BTB outcome for the selected animals was examined for the period 2002-2005, cattle that had a risk move were less likely to either fail a tuberculin test, or show confirmed factory lesion(s) at slaughter than those without a risk move (0.52% and 0.97% respectively).

The BTB risk associated with purchasing cattle during a herd restriction

Higgins, I.[1], Clegg, T.A.[1], Valy, L.[2], More, S.J.[1]

[1] UCD CVERA, [2] Ecole Nationale Vétérinaire de Toulouse, Toulouse, France

In Ireland, herds are restricted from trading for a (defined) period after TB has been detected. In some of these cases, the purchasing of animals into the restricted herd is allowed under permit to replace animals (production units) that were lost as a result of TB or to enable routine management practices (such as winter feeding of calves) to continue 'as normal' (in spite of the TB restrictions imposed). Concern has been raised as to whether this practice extends the restriction period.

Therefore, the objectives of this study are three-fold:
• To determine whether the purchasing of animals into a restricted herd is associated with increased TB risk
• To provide an overview of events associated with each purchasing episode and to clarify the infection status of animals that are introduced during restrictions
• If there is evidence of an increased TB risk, to identify the practices relating to the purchasing of animals that are the most risky and to determine whether the increased risk is associated with the source herd or the purchasing herd under restriction

To date, data has been extracted to identify the eligible population that were not restricted at the start of 2006, but experienced a TB episode during that year. Herds with unusual patterns of trading were excluded. Preliminary data analyses have been performed and further work is necessary to determine:
• Which practices relating to purchasing of animals during a restriction are the most risky, and
• Whether there is an increased risk associated with the source herd, the purchasing herd under restriction or both.
Two outcomes of interest have been identified, including duration of restriction (based on start and end dates) and time from de-restriction to subsequent breakdown or end of study [31DEC08], whichever occurred first.

Disentangling the relative importance of residual infection, contiguous spread and locality

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

Olea-Popelka, F.P.[1, 2], Costello, E.[3], White, P.[4, 5], McGrath, G.[5], Collins, J.D.[5], O'Keeffe, J.[4, 5], Kelton, D.F.[1], Berke, O.[1], More, S.[5], Martin, S.W.[1]

[1] Department of Population Medicine, Ontario Veterinary College, University of Guelph, Ontario, Canada, [2] Department of Clinical Sciences, College of Veterinary Medicine and Biomedical Sciences, Colorado State University, Colorado, USA, [3] Central Veterinary Research Laboratory, Department of Agriculture, Fisheries and Food, [4] Department of Agriculture, Fisheries and Food, [5] UCD CVERA

All the Irish cattle herds considered "clear" of bovine tuberculosis (BTB) having a single animal with a tuberculous lesion at slaughter during 2003 were identified. We performed a descriptive and logistic regression analysis to investigate whether selected risk factors had an association with the result of the herd test immediately after the tuberculous lesion was found ("Factory Lesion Test", FLT). At the FLT, only 19.7% (n = 338) of these 1713 herds had 1 or more standard reactors. The lesioned animal was home-bred in 46% of the "source" herds; these herds had an increased risk (23.4%) of having at least 1 standard reactor animal relative to herds with a purchased-lesioned animal (16.6%) (RR = 1.41). Our logistic models identified a number of important risk factors; two that appeared most important in predicting the FLT outcome were the time spent (residency) by the lesioned animal in the "source" herd, and the presence, or not, of the lesioned animal in a previous BTB episode in either the "source" herd, or the seller's herd in the case the lesioned animal was purchased. Our models fit the data well based on the Hosmer–Lemeshow test, however their sensitivity and specificity were very low (57% and 61% respectively). Surveillance of the cattle population for BTB using lesions found at slaughter is an essential component of an overall control program. Nonetheless, due to the poor predictability of the variables we measured, complete herd investigations are needed to help explain the FLT outcome of a herd.

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

White, P.[1, 2], Frankena, K.[3], O'Keeffe, J.[1, 2], More, S.J.[2], de Jong, M.[3], Martin, S.W.[4]

[1] Department of Agriculture, Fisheries and Food, [2] UCD CVERA, [3] Wageningen Institute of Animal Sciences, Wageningen University, The Netherlands,

[4] Department of Population Medicine, Ontario Veterinary College, University of Guelph, Ontario, Canada

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, these 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.

An evaluation of the Irish single reactor breakdown protocol for 2005 to 2008 and its use as a monitor of tuberculin test performance

Good, M.[1], Duignan, A.[1,2]

[1] Department of Agriculture, Fisheries and Food, [2] UCD CVERA

Under the Irish Bovine Tuberculosis (BTB) Eradication Programme, all herds are subjected to at least one test per annum. The Single Intra-dermal Comparative Tuberculin Test (SICTT) is used in Ireland for the detection of cattle infected with *Mycobacterium bovis*. There have been concerns regarding the specificity of the SICTT, notably by farmers, and particularly in herds where the detection of a single positive animal in the absence of an obvious source of (BTB) infection could be perceived as a "false" positive. To address this issue, the so-called 'Singleton Protocol' was established as part of the Irish BTB eradication programme. This protocol allows for the early restoration of free trading status to herds where a single positive animal was detected and where the herd was not confirmed as infected with *M. bovis* by epidemiological investigation, by *post mortem*, by laboratory examination, or by further test. This paper presents:

- data from the 2005 to 2008 BTB programmes on the number of herds which were assessed and which qualified for inclusion under the 'Singleton Protocol', and
- the outcome for qualifying herds up to and including having status restored early as a consequence of inclusion in that programme.

The outcome of this protocol reaffirms the reliability of the SICTT at current levels of infection. However, as overall infection levels of BTB fall, it is advocated that the 'Singleton Protocol' be continued as a monitor of herds in which a single positive animal is disclosed, to assess progress towards BTB eradication in Ireland.

Future risk of *Mycobacterium bovis* infection following an inconclusive diagnosis

Clegg, T.A.[1], Good, M.[2], Duignan, A.[1,2], Doyle, R.[2], More, S.J.[1]

[1] UCD CVERA, [2] Department of Agriculture, Fisheries and Food

A previous study has found that the risk of an animal being removed as a reactor was greater at an inconclusive reactor retest (91.9 reactors per 1000 animals tested) compared to the annual test (1.5 animals per 1000 animals tested). The cause of the higher reactor rate at an inconclusive reactor retest could be either due to the animals being truly infected or to a propensity for the tester to be "risk-averse" towards these animals. The objective of this study is to follow inconclusive reactor animals over their lifetime. Three specific time periods will be examined:

- the time from the inconclusive diagnosis to the inconclusive retest;
- the results of the inconclusive retest; and
- the time from retest until slaughter or the latest SICTT result.

In particular, we will examine whether inconclusives are more likely to be slaughtered prior to the inconclusive reactor retest, have lesions following a positive inconclusive reactor retest, be sold to another herd or slaughtered in the immediate period following a negative inconclusive reactor retest, fail the SICTT at some point in the future and/or have detectable *Mycobacterium bovis* infection at slaughter.

The risk of a positive test for bovine tuberculosis in cattle purchased from herds with and without a recent history of bovine tuberculosis in Ireland

Wolfe, D.M.[1], Berke, O.[1], More, S.J.[2], Kelton, D.F.[1], White, P.W.[2, 3], O'Keeffe, J.J.[2, 3], Martin, S.W.[1]

[1] Department of Population Medicine, Ontario Veterinary College, University of Guelph, Ontario, Canada, [2] UCD CVERA, [3] Department of Agriculture, Fisheries and Food

A retrospective cohort study was conducted to assess if cattle sold from Irish dairy herds within 7 months of herd de-restriction (clearance to trade) from a bovine-tuberculosis (BTB) episode had an excess risk of testing positive for BTB during the following 2 years, and to determine other risk factors associated with this outcome. If possible, a predictive metric for herds at high risk of selling future BTB-positive cattle would be generated. The unexposed cohort included all cattle sold within 7 months of the annual herd test in a random sample of dairy herds that did not test positive for BTB in 2003. The exposed cohort consisted of all cattle sold within 7 months of the date of de-restriction in all dairy herds that cleared a BTB episode in 2003. Only cattle sold from herds that were initially found to test positive for BTB using the single intradermal comparative tuberculin test (SICTT)—and not due to discovery of a BTB-positive animal at slaughter—were included as exposed cattle. To aid in the development of a predictive metric, the exposed cohort was subcategorized based on the number of reactors to the SICTT in the herd of origin during the BTB episode immediately prior to sale. The final exposure categories of 0 (unexposed), 1–7, and >=8 total reactors were considered the unexposed, mildly exposed, and severely exposed cohorts, respectively. A multivariable logistic regression model was fit to the final BTB status of the animal using a generalized estimating equation method (GEE), assuming an exchangeable correlation structure of animals within herds, and using robust standard errors. Exposure level and the other available herd- and animal-level information were modeled. After controlling for other risk factors including the size of the herd of origin and the sex and age of the animal, the three-level exposure variable significantly improved the model (based on a change in Quasi-Akaike Information Criteria of 2.2) and demonstrated a trend of increasing risk of a future positive BTB test with increasing exposure category. The severely exposed cohort of animals had significantly higher risk of a future positive BTB test than the unexposed cohort (OR = 1.78, p = 0.030).

Predictive models

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

Wolfe, D.M.[1], Berke, O.[1], Kelton, D.F.[1], White, P.W.[2, 3], More, S.J.[3], O'Keeffe, J.J.[2, 3], Martin, S.W.[1]

[1] Department of Population Medicine, Ontario Veterinary College, University of Guelph, Ontario, Canada,

[2] Department of Agriculture, Fisheries and Food, [3] UCD CVERA

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

A case control study of temporal and spatial risk factors associated with bovine tuberculosis breakdown herds in Irish cattle herds in 2006

White, P.[1, 2], Frankena, K.[3], O'Keeffe, J.[1, 2], More, S.J.[1], de Jong, M.[3]

[1] Department of Agriculture, Fisheries and Food, [2] UCD CVERA, [3] Wageningen Institute of Animal Sciences, Wageningen University, The Netherlands

Although the temporal-spatial distribution (clustering) of BTB breakdown herds are well recognised in Ireland, and also the chronic nature of BTB infection within individual herds, little work has been published to date on the persistence of BTB infection within neighbourhoods. Using a logistic regression model, this paper aims to quantify the effect of the BTB infection status in neighbouring herds on the risk of new BTB outbreaks, while controlling for potential confounding factors. Based on 2006 test data, herds experiencing a breakdown with at minimum 1 standard reactor are classed as case herds, while herds that remained unrestricted throughout 2006 are classed as control herds. Neighbouring herds within 1 km of each study herd, will be zoned according to Euclidean distance to the nearest related fragment (within 25 m, 26-150 m, 151-500 m, and 501-1,000 m of a study herd) based on land parcel claims for 2005. BTB herd history of neighbouring herds within each zone will be assessed over the period 1989-2005 as a potential risk factor for a herd breakdown in 2006. Other herd factors will be controlled for within the model, including own herd BTB history over the period 1989-2005, purchasing history, herd size, and enterprise type. The predictive value of various competing models will be compared, and using a final model and an assessment made of the contribution of neighbourhood factors to future BTB risk.
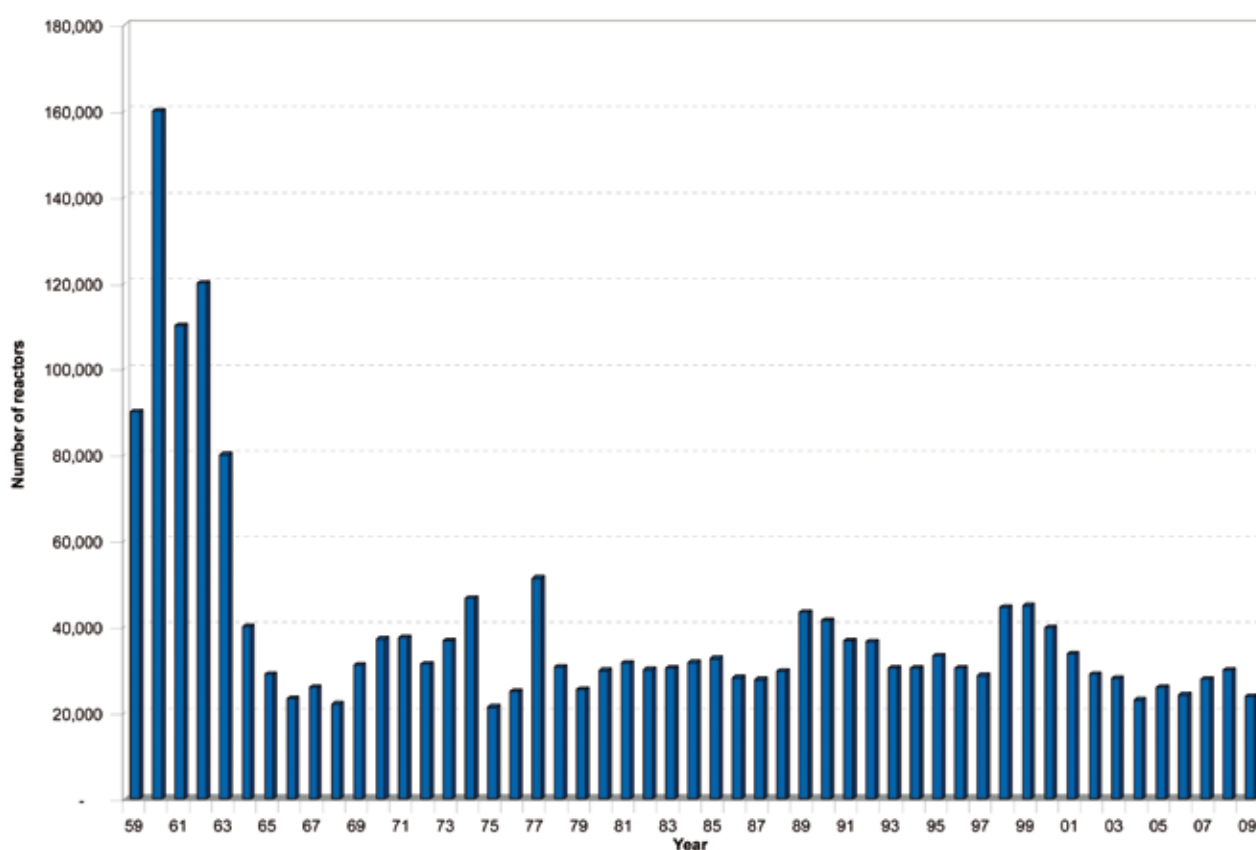
The number of TB reactors detected in Ireland each year between 1959 and 2009.

Supporting studies

Genetics, milk production

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

Brotherstone, S.[1], White, I.M.S.[1], Coffey, M.[2], Downs, S.H.[3], Mitchell, A.P.[3], Clifton-Hadley, R.S.[3], More, S.J.[4], Good, M.[5], Woolliams, J.A.[6]

[1] School of Biological Sciences, University of Edinburgh, Scotland, [2] Scottish Agricultural College, Midlothian, Scotland, [3] Veterinary Laboratories Agency, Weybridge, Surrey, United Kingdom, [4] UCD CVERA, [5] Department of Agriculture, Fisheries and Food, [6] The Roslin Institute, Midlothian, Scotland

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

Genetics of tuberculosis in Irish Holstein-Friesian dairy herds

Bermingham, M.L.[1], More, S.J.[2], Good, M.[3], Cromie, A.R.[4], Higgins, I.[2], Brotherstone, S.[5], Berry, D.P.[1]

[1] Teagasc Moorepark Production Research Centre, [2] UCD CVERA, [3] Department of Agriculture, Fisheries and Food,

[4] Irish Cattle Breeding Federation Society Ltd., [5] Institute of Evolutionary Biology, University of Edinburgh, Edinburgh, Scotland

Information is lacking on genetic parameters for tuberculosis (TB) susceptibility in dairy cattle. *Mycobacterium bovis* is the principal agent of tuberculosis in cattle. The objective of this study was to quantify the genetic variation present among Irish Holstein- Friesian dairy herds in their susceptibility to *M. bovis* infection. A total of 15,182 cow and 8,104 heifer single intradermal comparative tuberculin test (SICTT, a test for *M. bovis* exposure and presumed infection) records from November 1, 2002, to October 31, 2005, were available for inclusion in the analysis. Data on observed carcass TB lesions from abattoirs were also available for inclusion in the analysis. The only animals retained were those present in a herd during episodes in which at least 2 animals showed evidence of infection; this ensured a high likelihood of exposure to *M. bovis*. Linear animal models, and sire and animal threshold models were used to estimate the variance components for susceptibility to *M. bovis*-purified protein derivative (PPD) responsiveness and confirmed *M. bovis* infection. The heritability estimates from the threshold sire models were biased upward because the relatedness between dam-daughter pairs was ignored. The threshold animal model produced heritability estimates of 0.14 in cows and 0.12 in heifers for susceptibility to *M. bovis*-PPD responsiveness, and 0.18 in cows for confirmed *M. bovis* infection susceptibility. Therefore, exploitable genetic variation exists among Irish dairy cows for susceptibility to *M. bovis* infection. Sire

rankings from the linear and threshold animal models were similar, indicating that either model could be used for the analysis of susceptibility to *M. bovis*-PPD responsiveness. A favorable genetic correlation close to unity was observed between susceptibility to confirmed *M. bovis* infection and *M. bovis*-PPD responsiveness, indicating that direct selection for resistance to *M. bovis*-PPD responsiveness will indirectly reduce susceptibility to confirmed *M. bovis* infection. Data from the national TB eradication program could be used routinely to estimate breeding values for susceptibility to *M. bovis* infection.

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

Bermingham, M.L.[1], More, S.J.[2], Good, M.[3], Cromie, A.R.[4], Higgins, I.M.[2], Berry, D.P.[1]

[1] Teagasc Moorepark Production Research Centre, [2] UCD CVERA, [3] Department of Agriculture, Fisheries and Food,

[4] Irish Cattle Breeding Federation Society Ltd.

Previous research has shown that there is considerable genetic variation for susceptibility to the measures of *Mycobacterium bovis* infection, confirmed *M. bovis* infection and *M. bovis*-purified protein derivative (PPD) responsiveness in Irish Holstein-Friesian dairy cattle. The objective of this study was to estimate the genetic and phenotypic correlations between economically important traits and these measures of *M. bovis* infection. A total of 20,148 and 17,178 cows with confirmed *M. bovis* infection and *M. bovis*-PPD responsiveness records respectively were available for inclusion in the analysis. First to third parity milk, fat, and protein yield, somatic cell count, calving interval and survival, as well as first parity body condition score records were available on cows calving between 1985 and 2007. Bivariate linear sire mixed models were used to estimate (co)variance components. The genetic correlations between susceptibility to confirmed *M. bovis* infection and economically important traits investigated in this study were all close to zero. Susceptibility to *M. bovis*-PPD responsiveness was positively genetically correlated with fat production (0.39) and body condition score (0.36), and negatively correlated with somatic cell score (-0.34) and survival (-0.62). Hence, selection for increased survival may indirectly reduce susceptibility to *M. bovis* infection, while selection for reduced somatic cell count and increased fat production and body condition score may increase susceptibility to *M. bovis* infection.

Bovine tuberculosis and milk production in infected dairy herds in Ireland

Boland, F.[1], Kelly, G.E.[1], Good, M.[2], More, S.J.[3]

[1] UCD School of Mathematical Sciences, [2] Department of Agriculture, Fisheries and Food, [3] UCD CVERA

This study describes the relationship between bovine tuberculosis (TB) and milk yield in TB-infected dairy herds in Ireland. The study had two objectives: to determine whether cows detected as TB reactors (and thus subject to immediate slaughter) were likely to be the higher milk-producing cows, and to determine whether subclinical TB infection was associated with reduced milk production at or around the time of disclosure (detection). All Irish dairy herds restricted from trading between the 1st June 2004 and the 31st May 2005 as a result of two or more TB reactors by the Single Intradermal Comparative Tuberculin Test (SICTT) were considered for study. The data consisted of 419 herds. Data were collected on all TB reactors and a random sample of 5 non-reactor cows in these herds: a data set of 4340 cows (2342 TB reactors and 1998 non-reactors). Previous milk data for the cows were taken into consideration and thus all lactations on a cow were analysed together with the years of lactations. There was an inherent hierarchical structure in the data, with lactations nested within cows and cows within herds and thus a linear mixed model with two random effects was used to describe the data. The results of this study showed that for all lactations and years under investigation, milk yield was significantly lower for TB reactor cows, with differences ranging from 120 kg (2003, lactation 3) to 573 kg (2001, lactation 1), when compared to the non-reactor cows.

Tuberculosis in other farmed livestock species

Control of *Mycobacterium bovis* infection in two sika deer herds in Ireland

Partridge, T.[1], Toolan, D.[2], Egan, J.[1], More, S.J.[3]

[1] Department of Agriculture, Fisheries and Food, [2] Kilkenny Regional Veterinary Laboratory, Department of Agriculture, Fisheries and Food, [3] UCD CVERA

In a number of countries, tuberculosis (due to infection with *Mycobacterium bovis*) is a significant health problem of captive deer. This paper describes outbreaks of bovine tuberculosis in sika deer *(Cervus nippon)* on two farms in Ireland and the methods used to control the disease. On Farm A, infection was first detected during 1993. The infection was eradicated using a programme of test and removal, in association with segregation of young animals. A second outbreak (also due to infection with *M. bovis*, but a different RFLP profile) was detected in 2002. In the latter outbreak, infection was particularly prevalent in two groups of young deer. *M. bovis* with the same RFLP profile was also isolated in a badger found dead on the farm. Control was achieved by test and removal in association with herd management changes. In Herd B, infection was first detected in 1995, and subsequently eradicated using test and removal alone. In herd A, re-infection remains an ongoing risk. Control rather than eradication of infection may be more realistic in the short- to medium-term.

Tuberculosis in alpaca *(Lama pacos)* on a farm in Ireland. 1. A clinical report

Ryan, E.G.[1], Dwyer, P.J.[2], Connolly, D.J.[3], Fagan, J.[2], Costello, E.[4], More, S.J.[5]

[1] Private veterinary practitioner, [2] Athlone Regional Veterinary Laboratory, Department of Agriculture, Fisheries and Food,

[3] Private consultant, [4] Central Veterinary Research Laboratory, Department of Agriculture, Fisheries and Food, [5] UCD CVERA

This case report describes tuberculosis (TB) due to infection with *Mycobacterium bovis* in alpaca *(Lama pacos)* on a farm in Ireland. Two severely debilitated alpaca were presented to the University Veterinary Hospital, University College Dublin in November 2004. Bloods were taken, and haematology and biochemistry results were indicative of chronic infection. Radiological examination showed evidence of diffuse granulomatous pneumonia suggestive of tuberculosis. On necropsy there were granulomatous lesions present throughout many body organs including lung, liver, kidney, intestine as well on peritoneum and mesentery. Culture of acid-fast bacilli from lesions led to a diagnosis of tuberculosis due to *M. bovis*. The use of intradermal skin testing proved inefficient and unreliable for ante mortem diagnosis of tuberculosis in alpaca. Infection due to *M. bovis* should be considered among the differential diagnoses of debilitating diseases in alpaca, particularly those farmed in areas known to be traditional black spots for tuberculosis in cattle.

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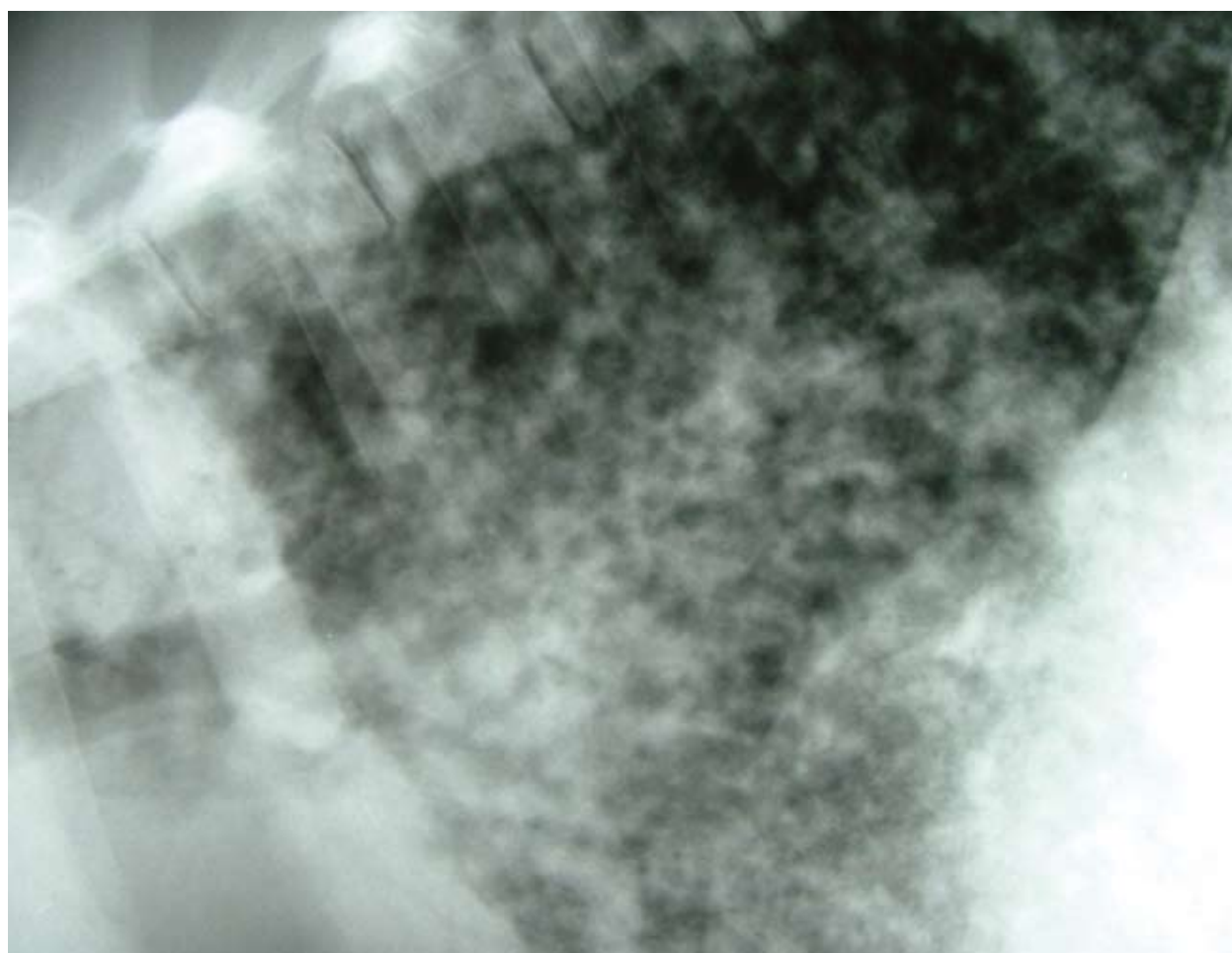
Lateral radiograph (x-ray) from an alpaca with pulmonary tuberculosis. Radiograph taken at the UCD Veterinary Hospital.

Tuberculosis in alpaca *(Lama pacos)* on a farm in Ireland. 2. Results of an epidemiological investigation

Connolly, D.J.[1], Dwyer, P.J.[2], Fagan, J.[2], Hayes, M.[3], Ryan, E.G.[4], Costello, E.[5], Kilroy, A.[3], More, S.J.[6]

[1] Private consultant, [2] Athlone Regional Veterinary Laboratory, Department of Agriculture, Fisheries and Food, [3] Department of Agriculture, Fisheries and Food, [4] UCD School of Agriculture, Food Science and Veterinary Medicine, [5] Central Veterinary Research Laboratory, Department of Agriculture, Fisheries and Food, [6] UCD CVERA

Tuberculosis (TB), due to infection with *Mycobacterium bovis* was diagnosed in a flock of alpaca in Ireland in 2004. An epidemiological investigation was conducted to identify the risk of TB for farmed alpaca where TB is endemic, the origin of the infection, the potential for alpaca-to-alpaca transmission and appropriate control measures. The investigation focused on the alpaca flock (including the farm, animal movements and breeding, feeding and flock health practice), the disease episode (including animal disease events and subsequent control measures) and TB infection risk in the locality. The TB risk to alpaca is high in areas where infection is endemic in cattle and badgers and where biosecurity is inadequate. It is most likely that the source of infection for the alpaca was a local strain of *M. bovis*, present in cattle in this area since at least 2001. Genotyping of isolates identified a single variable number tandem repeat (VNTR) profile in both cattle and alpaca in this region. Although a tuberculous badger was also removed from the vicinity, bacterial isolation was not attempted. On this farm, infection in alpaca was probably derived from a common source. Alpaca-to-alpaca transmission seems unlikely. Two broad control strategies were implemented, aimed at the rapid removal of infected (and potentially infectious) animals and the implementation of measures to limit transmission. Tests that proved useful in detecting potentially-infected animals included measurement of the albumin-to-globulin ratio and regular body condition scoring. Skin testing was time consuming and unproductive, and early detection of infected animals remains a challenge. The flock was managed as a series of separate groupings, based on perceived infection risk. No further TB cases have been detected.

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

Shanahan, A.[1], Good, M.[1], Duignan, A.[1, 2], Curtin, T.[1], More, S.J.[2]

[1] Department of Agriculture, Fisheries and Food, [2] UCD CVERA

In a number of countries, bovine tuberculosis (BTB; due to infection with *Mycobacterium bovis*) is a significant health problem of farmed goats. However, few case reports are available. In Ireland, BTB is a problem in the national cattle herd, and is endemic in badgers *(Meles meles)*. Infection has also been periodically confirmed in goats. Under EU Regulation 853/2004/EC, Ireland requires food business operators collecting or producing raw milk from species susceptible to BTB, which includes goats, to have a control plan for tuberculosis in place and approved by the competent Authority. This paper describes an outbreak of bovine tuberculosis in a dairy goat herd on a farm in Ireland, where 66.3% of the herd was SICTT-positive at initial detection. An epidemiological investigation was conducted to determine the origin of the outbreak. The investigation considered a range of issues including animal movements and herd management practices. Infection was introduced to the holding with a consignment of goats as determined by the VNTR (variable number tandem repeat) profile. The infection was eradicated using a programme of test and removal involving the SICTT (single intradermal comparative tuberculin test), the γ-interferon assay and a multiplex immunoassay (Enferplex TB). These tests are still at development stage for use in goats in Ireland. There was good correlation between tests, and infection was rapidly cleared from this heavily infected herd.

Alpaca, the west of Ireland. Photograph by S.J. More.

Improved understanding of ecology and TB epidemiology

Badger ecology

How many Eurasian badgers *Meles meles* L. are there in the Republic of Ireland?

Sleeman, D.P.[1], Davenport, J.[1], More, S.J.[2], Clegg, T.A.[2], Collins, J.D.[2], Martin, S.W.[3], Williams, D.H.[4], Griffin, J.M.[5], O'Boyle, I.[5]

[1] Department of Zoology, Ecology and Plant Science, University College Cork, [2] UCD CVERA, [3] Department of Population Medicine, Ontario Veterinary College, University of Guelph, Ontario, Canada, [4] UCD School of Mathematical Sciences, [5] Department of Agriculture, Fisheries and Food

In Ireland, the badger *Meles meles* L. is a reservoir species for *Mycobacterium bovis* and, as such, contributes to the maintenance of bovine tuberculosis in cattle. A previous estimate of the badger population in the Republic was 200,000 badgers. In the current study, we obtained data on badger numbers from a large-scale badger removal project (the Four-Area project). The removal areas of the Four-Area Project were surrounded by barriers (either water or buffer areas where removals were also conducted) to prevent badger immigration. Within these areas, a grid of 0.25 km^2 was created within which we knew the badger numbers and habitat types (based on Corine data). Associations between badger numbers and habitat type were investigated using negative binomial modeling. Extrapolations from the model yielded an estimated badger population in the Republic of approximately 84,000 badgers. The implications of these findings are discussed.

The effectiveness of barriers to badger *Meles meles* immigration in the Irish four area project

Sleeman, D.P.[1], Davenport, J.[1], More, S.J.[2], Clegg, T.A.[2], Griffin, J.M.[3], O'Boyle, I.[3]

[1] Department of Zoology, Ecology and Plant Science, University College Cork, [2] UCD CVERA, [3] Department of Agriculture, Fisheries and Food

This study's objective was to estimate the permeability of barriers to badger immigration during the Irish Four Area project. These barriers were at the boundaries of removal areas, where there was proactive culling of badgers. Data from the last 3 years of the study were used. Each length of barrier was allocated a space within the removal area. These were further sub-divided into spaces of 0–2, 2–5 km and sometimes of more than 5 km from the edge of the removal area. It is assumed that all, or some, of the badgers caught within these spaces came across the barriers. The barriers were one of the following: external buffers, sea, rivers and political boundaries. The total lengths of the barriers in all areas were: external buffer 128.5 km; sea 70.9 km; river 78.6 km; political 32.2 km. We assume three scenarios: (1) all badgers caught in the final 3 years were immigrants, (2) 75% were immigrants or (3) 50% were immigrants. We test these scenarios using chi-square tests, applying internal buffers of 1 km to counter movements of badgers across zones. Using this approach and multivariate analysis, we found that the permeability of barrier types varied, with sea and external buffers being the most effective barriers. The combined capture data are further examined by the sex ratio in each range, and then the sex ratio in total. Equal numbers of males and females were found, but the source populations were probably predominantly female. If badger management options are to achieve maximum benefits, then the field effectiveness of such barriers needs to be understood.

The small-bodied badgers *(Meles meles L.)* of Rutland Island, Co. Donegal

Sleeman, D.P.[1], Davenport, J.[1], Cussen, R.E.[1], Hammond, R.F.[2]

[1] Department of Zoology, Ecology and Plant Science, University College Cork, [2] UCD CVERA

Badgers found on an island, Rutland Island, Co. Donegal were investigated. When compared with badgers from the Donegal mainland they are significantly smaller in both length and weight. Twenty-eight setts were found in a survey in 1998 and fifteen Badgers were trapped in 1999. GPS was used to map and re-locate setts and latrines. Bait-marking in 2003 showed that there were two social groups on the island at that time. The results are discussed with reference to the badgers' adaptation to the island, their population structure, use of setts and the research opportunities, for both badger management and conservation, on the island.

The diet of the badger *Meles meles* in the Republic of Ireland

Cleary, G.P.[1], Corner, L.A.L.[2], O'Keeffe, J.[3, 4], Marples, N.M.[1]

[1] School of Natural Sciences, Trinity College Dublin, [2] UCD TB Diagnostics and Immunology Research Centre,

[3] Department of Agriculture, Fisheries and Food, [4] UCD CVERA

The diet of the Eurasian badger *(Meles meles)* in the Republic of Ireland was studied by examination of the stomach of the contents of 686 badgers, collected between March 2005 and September 2006. It was found that the relative importance of different food types, as indicated by their frequency of occurrence and ingested bulk in the diet, fluctuated seasonally. Tipulid larvae (Cl. Insecta, Ord. Tipulidae) dominated the diet in spring; Anura (Cl. Amphibia) and Aculeata (Ord. Hymenoptera) during the summer; and Noctuid larvae (Cl. Insecta, Ord. Noctuidae) in autumn and winter. Thus this type of foraging behaviour supports the contention that badgers are generalist foragers with seasonal food preferences. This feeding behaviour is more similar to that of badgers in Italy and Spain than to badgers in England.

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

Cleary, G.P.[1], Corner, L.A.L.[2], O'Keeffe, J.[3, 4], Marples, N.M.[1]

[1] School of Natural Sciences, Trinity College Dublin, [2] UCD TB Diagnostics and Immunology Research Centre,

[3] Department of Agriculture, Fisheries and Food, [4] UCD CVERA

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

The reproductive cycle of the male and female Eurasian badger

Stuart, L.[1], Corner, L.A.L.[2], O'Keeffe, J.[3, 4], Marples, N.M.[1]

[1] School of Natural Sciences, Trinity College Dublin, [2] UCD TB Diagnostics and Immunology Research Centre,

[3] Department of Agriculture, Fisheries and Food, [4] UCD CVERA

The nationwide control programme of reactive badger removal provided an excellent opportunity to detail the reproductive cycle of the male and female badger in Ireland, as a comparison to previous studies from England and parts of mainland Europe. Badgers were obtained from a geographically wide range of sites in Ireland during all months of the year, making it possible to describe the annual reproductive cycle of the male and female badger in full, utilising a large sample size. The reproductive tracts were obtained from post mortem examinations of the badger. Both gross examination and in-depth histological examinations were carried out on all tissues; assays were conducted to determine individual levels of reproductive hormones. A number of demographic measurements were also taken to help with identification of trends or varying population dynamics within the study population. The badger population in Ireland is of low-medium density consisting of small social groups. The lack of aggression observed suggests that there may be high levels of tolerance both within and between social groups; possibly owing to small group sizes, high levels of philopatry, inter-group relatedness, and relatively low levels of testosterone. Mate guarding behaviour may be abandoned due to high levels of philopatry or an inability to monopolise paternity. A comparison of male and female badgers suggests that each has adopted a very different breeding strategy. The majority of males show a breeding pattern that is reminiscent of a seasonal breeder, with high fertility associated with the early oestrous cycles of the female in Feb-April and declining fertility for the remainder of the year. By contrast the female has adopted a strategy whereby they are lactating during a period of nutritional abundance in Feb-March, and cubs are also weaned during favourable conditions. This is achievable due to the long period of delayed implantation employed by this species, such that implantation takes place in Dec-Jan. Badgers have continued oestrous cycles throughout the breeding year, providing replacement or additional blastocysts, which increases the probability of successful conception at the end of the period of delay. Furthermore, continued oestrous cycles may provide additional corpora lutea as a source of progesterone, needed to sustain the diapausing blastocysts. This female strategy also increases the probability of superfetation and polyandry which leads to an increase in female fitness and increased cub survival. Approximately 40% of mature females bred successfully and as the number of blastocysts per female was similar to the number of foetuses, the potential was maintained through to parturition. Failure at the fertilisation stage of the reproductive cycle was responsible for the greatest losses to reproductive potential.

The effects of culling on the badger *(Meles meles)* population in Ireland

Carroll, R.[1], Corner, L.A.L.[2], Marples, N.M.[1]

[1] School of Natural Sciences, Trinity College Dublin, [2] UCD TB Diagnostics and Immunology Research Centre

Since the interim ban on culling implemented by the Department of Agriculture, Food and Fisheries (DAFF) is set to continue, it is essential that it takes place at the most appropriate time of year. If not then there is a danger of suckling females being captured when they still have dependent cubs, thus leaving the cubs to starve. When determining the most opportune time for this closed season to take place it is important to establish whether cubbing occurs at the same time each year and if not, then what factors affect the birthing dates.

The information gathered from the postmortems carried out on these badgers enabled us to describe the cubbing season of badgers in Ireland, and so prevent the culling of suckling sows. Therefore the first aim of this project was to predict the annual cubbing trends in Ireland and investigate the differences in social group size and reproductive success found between areas of previously undisturbed groups and newly established groups. The second aim was to investigate whether the bovine TB (bTB) eradication scheme has changed the badger population in Ireland with regard to social group organisation and reproductive potential. We further aimed to investigate social group dynamics, focusing mainly on sett densities and usage, and recovery times after culling. Finally we hope to develop a methodology which will successfully age a subsection of the Irish badger population, using teeth sectioning and x-ray imaging techniques, using both known and unknown age jaws.

Incidence of visits by badgers to farmyards in Ireland in winter

Sleeman, D.P.[1], Davenport, J.[1], Fitzgerald, A.[2]

[1] Department of Zoology, Ecology and Plant Science, University College Cork, [2] Department of Epidemiology and Public Health, University College Cork

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 quantified the badger activity in cattle yards in Co. Cork. It was found to be very rare.

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Mammalian road casualties in the Cork four area project

O'Shea, F.[1], Sleeman, D.P.[2], Davenport, J.[2]

[1] Department of Agriculture, Fisheries and Food, [2] Department of Zoology, Ecology and Plant Science, University College Cork

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

TB epidemiology

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

Kelly, G.E.[1], McGrath, G.E.[2], More, S.J.[2]

[1] School of Mathematical Sciences, University College Dublin, [2] UCD CVERA

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

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

Murphy, D.[1], Gormley, E.[1], Costello, E.[2], O'Meara, D.[2], Corner, L.A.L.[1]

[1] UCD TB Diagnostics and Immunology Research Centre, [2] Central Veterinary Research Laboratory, Department of Agriculture, Fisheries and Food

The accurate diagnosis of *Mycobacterium bovis* infection in badgers is key to understanding the epidemiology of tuberculosis in this species and has significant implications for devising strategies to limit spread of the disease. In this study, badgers (n = 215) in the Republic of Ireland were examined at post mortem and tissues were collected from a range of anatomical locations and pooled into groups for bacterial culture of *M. bovis*. By assessing confirmed gross visible lesions (VL) alone, infection was detected in 12.1% of badgers. However, by including the results of all culture positive pooled samples, the overall infection prevalence increased significantly to 36.3%. Two-thirds (66.7%) of infected animals had no visible lesions (NVL). While the thoracic cavity (lungs and pulmonary lymph nodes) was found to be the most common site of infection, in a proportion of animals infection was absent from the lungs and draining lymph nodes and was confined to the lymph nodes of the carcase or the head. This may indicate an early extra-pulmonary dissemination of infection or alternatively, in the case of the head lymph nodes, a secondary pathogenic pathway involving the lymphoid tissues of the upper respiratory tract (URT).

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

Murphy, D.[1], Gormley, E.[1], Collins, D.M.[2], McGrath, G.[2], Sorvic, E.[3], Costello, E.[3], Corner, L.A.L.[3]

[1] UCD TB Diagnostics and Immunology Research Centre, [2] UCD CVERA,

[3] Central Veterinary Research Laboratory, Department of Agriculture, Fisheries and Food

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

The Irish redfield study

Murphy, D.[1], Gormley, E.[1], Collins, D.M.[2], McGrath, G.[2], Sorvic, E.[3], Costello, E.[3], Corner, L.A.L.[3]

[1] UCD TB Diagnostics and Immunology Research Centre, [2] UCD CVERA,

[3] Central Veterinary Research Laboratory, Department of Agriculture, Fisheries and Food

In parallel to the Greenfield study, a study has being conducted of badgers removed from routine first event culls associated with herd breakdowns. The Redfield study examines the infection prevalence in badgers removed in the first trapping event in areas of high prevalence of infection in cattle. Bacterial culture results are pending. 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.

Spatial clustering of TB-infected cattle herds in Ireland prior to and following pro-active badger removal

Kelly, G.E.[1], More, S.J.[2]

[1] School of Mathematical Sciences, University College Dublin, [2] UCD CVERA

Bovine tuberculosis (TB; caused by infection with *Mycobacterium bovis*) is primarily a disease of cattle. In both Ireland and the UK, badgers *(Meles meles)* contribute to the epidemiology of infection in cattle. Control of infection depends in part on our understanding of the spatial structure of the disease. Data from the Four Area Project, a large-scale intervention study aimed at assessing the effect of proactive badger culling on bovine TB incidence in cattle herds, are analyzed for the first time using logistic models that explicitly include spatial random effects i.e. generalized linear geostatistical models. We establish that infected herds are spatially correlated (the scale of spatial correlation is presented), but at a scale that varies with time and in different areas. Spatial correlation is shown to persist following proactive badger removal, consistent with ongoing residual herd infection. The results are useful in informing TB control policy.

Infection control strategies

Badger removal

Controlling wildlife reservoirs for bovine TB

Hewinson, G.[1], Buddle, B.[2], More, S.J.[3], Clifton-Hadley, R.[1]

[1] Veterinary Laboratories Agency, Weybridge, Surrey, England, [2] AgResearch, Hopkirk Research Institute, Palmerston North, New Zealand, [3] UCD CVERA

Bovine tuberculosis (bTB) is caused by *Mycobacterium bovis* and closely related members of the *M. tuberculosis*-complex. These organisms have an extensive host range that includes bovines, other livestock including small ruminants such as goats, a wide range of wildlife and humans. Eradication programmes based on cattle test-and-slaughter policy have proved successful in some countries but have failed to eradicate disease in others due, at least in part, to the presence of reservoirs of TB in wildlife. There are three main approaches to controlling the spread of TB from wildlife to livestock: keeping livestock and wildlife apart, culling or vaccination. However, the control of tuberculosis in wildlife is a complex process and involves an understanding of the mechanisms of transmission of *M. bovis* between wildlife, and from wildlife to livestock, as well as the ecology, biology and behavior of the wildlife reservoir. Without such knowledge implementation of control strategies can lead to unexpected consequences. Approaches to the control of TB in wildlife reservoirs will be discussed in the context of the successful Australian TB eradication scheme, TB control in possums in New Zealand and the badger culling trials that have been conducted in Great Britain.

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

Corner, L.A.L.[1], Clegg, T.A.[2], More, S.J.[2], Williams, D.H.[3], O'Boyle, I.[4], Costello, E.[5], Sleeman, D.P.[6], Griffin, J.M.[4]

[1] UCD TB Diagnostics and Immunology Research Centre, [2] UCD CVERA, [3] School of Mathematical Sciences, University College Dublin,

[4] Department of Agriculture, Fisheries and Food, [5] Central Veterinary Research Laboratory, Department of Agriculture, Fisheries and Food,

[6] Department of Zoology, Ecology and Plant Science, University College Cork

We examined the effect of varying levels of badger population control on the prevalence of *Mycobacterium bovis* infection in badgers in four counties of Ireland. In the 'Removal' and 'Buffer' areas, proactive culling was conducted to substantially reduce and subsequently maintain badger populations at a low level for five years. In the 'Reference' areas, localised reactive culling was conducted in association with herd breakdowns. The infection status of badgers was determined using bacteriology. A total of 2,696 badgers were recruited into the study, and 19.0% were found to be infected with *M. bovis*. The two population control strategies had differing effects on the subsequent prevalence of tuberculosis in badger populations. Proactive culling led to a long term decrease in the prevalence of tuberculosis in the re-emergent populations. Although there was an overall decline in the disease prevalence, no consistent trend in disease prevalence as a result of reactive culling was observed.

A long-term observational study of the impact of badger removal on herd restrictions due to bovine TB in the Irish midlands during 1989–2004

Kelly, G.E.[1], Condon, J.[2], More, S.J.[3], Dolan, L.[4], Higgins, I.[3], Eves, J.[4]

[1] School of Mathematical Sciences, University College Dublin, [2] School of Mathematical Sciences, Dublin Institute of Technology,

[3] UCD CVERA, [4] Department of Agriculture, Fisheries and Food

An observational study was carried out, using data collected from four areas in the Irish midlands, between 1989 and 2004, to critically evaluate the long-term effects of proactive badger culling and to provide insights into reactive badger culling tuberculosis (TB) prevalence in cattle. Confirmed cattle herd TB incidence is the outcome measure used throughout. Relative to reactive culling, proactive badger culling was associated with a decrease in incidence in each of the 16 years of observation, which encompassed periods of both intensive and less-intensive badger removal. By 2004, we observed a decrease of 22% [95% confidence interval (CI) 15–29, $P<0.001$] in the entire proactive and 37% (95% CI 25–47, $P<0.001$), in the inner proactive removal areas. The size of the decrease increased with time ($P=0.055$). There was a decrease (constant over time) of at least 14% (95% CI 76–97, $P=0.013$) in incidence in the inner compared to the outer control area (herds <=2 km, >2 km, from proactive removal area boundaries, respectively). Incidence in the outer proactive removal area (herds <1.6 km from the proactive removal boundary) was similar to the inner control area ($P=0.890$). Incidence in the outer control area and total control area, compared to a neighbouring area some distance away, increased over the course of the study. Differences with the total control area were not statistically significant but the outer control area was 11% higher than the neighbouring area by 2004 (borderline significance $P=0.057$).

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Targeted badger removal and the subsequent risk of bovine tuberculosis in cattle herds in county Laois, Ireland

Olea-Popelka, F.P.[1, 2], Fitzgerald, P.[3], White, P.[3, 4], McGrath, G.[4], Collins, J.D.[4], O'Keeffe, J.[3, 4], Kelton, D.F.[1], Berke, O.[1], More, S.J.[4], Martin S.W.[1]

[1] Department of Population Medicine, Ontario Veterinary College, University of Guelph, Ontario, Canada, [2] Department of Clinical Sciences, College of Veterinary Medicine and Biomedical Sciences, Colorado State University, Colorado, USA, [3] Department of Agriculture, Fisheries and Food, [4] UCD CVERA

We investigated the impact of targeted removal of badgers on the subsequent bovine tuberculosis (BTB) risk in cattle herds in county Laois, Ireland. The study period was 1989–2005. For each of 122 targeted badger-removal licenses (permit to remove badgers in the proximity of cattle herds undergoing a serious BTB episode), the herd number (index herd) for which the license was given was obtained. The herds in the proximity of the index herd were identified from another database. The main "exposure" in our study was the geographical location of herds relative to the area in which targeted badger removal was conducted. We categorized herds into five different exposure groups: herds were classified as non-exposed and denoted as group 0 (reference group) if they were located 500 m or more from the edge of any parcel of land of the index herd; group 1, was the index herds, group 2 the immediate (contiguous) neighbors of the index herd, group 3 herds were not immediate neighbors but within 150 m and group 4 herds were between 150 m and 500 m distance from the edge of any parcel of land of the index herd, respectively. We conducted a survival analysis (allowing multiple failures per herd) to compare the hazard of having a BTB episode in any of the four groups of exposed herds vs. the hazard in herds in the reference group. We controlled for other known risk factors as well taking into account a temporal component. Our analysis showed that the hazard ratio for the index herds (group 1) were non-significantly

increased, indicating that there was no difference in the hazard of failing a BTB test (after the targeted badger removal was conducted) between index herds and reference herds. For the rest of the herds farther away from badger removal activities the hazards were lower than herds in areas not under badger removal. The hazard in the reference group decreased over the study period.

This article was published in Preventive Veterinary Medicine, 88, Olea-Popelka, F.P., Fitzgerald, P., White, P., McGrath, G., Collins, J.D., O'Keeffe, J., Kelton, D.F., Berke, O., More, S.J., Martin S.W., Targeted badger removal and the subsequent risk of bovine tuberculosis in cattle herds in county Laois, Ireland, 178-184, Copyright Elsevier B.V. 2009.

The impact of targeted badger removal on tuberculosis in cattle herds in Co. Monaghan

White, P.[1, 2], Frankena, K.[3], O'Keeffe, J.[1, 2], More, S.J.[2], de Jong, M.[3], Martin, S.W.[4]

[1] Department of Agriculture, Fisheries and Food, [2] UCD CVERA, [3] Wageningen Institute of Animal Sciences, Wageningen University, The Netherlands,

[4] Department of Population Medicine, Ontario Veterinary College, University of Guelph, Ontario, Canada

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.

An assessment of injury to European badgers *(Meles meles)* due to capture in stopped restraints

Murphy, D.[1], O'Keeffe, J.J.[2, 3], Martin, S.W.[4], Gormley, E.[1], Corner, L.A.L.[1]

[1] UCD TB Diagnostics and Immunology Research Centre, [2] Department of Agriculture, Fisheries and Food, [3] UCD CVERA,

[4] Department of Population Medicine, Ontario Veterinary College, University of Guelph, Ontario, Canada

As part of ongoing culling operations, European badgers *(Meles meles)* were captured using stopped restraints in winter (October to December 2005) and summer (May to June 2006) in the Republic of Ireland. A subset of these badgers, those caught during four consecutive nights, was examined postmortem to determine the frequency and severity of physical injuries resulting from capture in the restraints. The skin and the tissues underlying the restraint of 343 badgers were assessed for injury by visual examination. There was an absence of skin damage or only minor skin abrasions in 88% of badgers; an absence of subcutaneous tissue injury or only localized subcutaneous tissue injury in 69%; and an absence of muscle injury or only slight muscle bruising in 99% of badgers. Only 2% of badgers had cuts to the skin and 5.5% had extensive subcutaneous edema, whereas 1.2% had areas of hemorrhage and tearing of the underlying muscle. Our results show that the majority of badgers examined sustained minimal injuries attributable to capture in stopped restraints.

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Progress towards a badger vaccine

Badger tuberculosis vaccine

Corner, L.A.L.[1], Murphy, D.[1], Costello, E.[2], Gormley, E.[1]

[1] UCD TB Diagnostics and Immunology Research Centre, [2] Central Veterinary Research Laboratory, Department of Agriculture, Fisheries and Food

The research on BCG vaccination against tuberculosis in captive badgers, an integrated series of experiments and associated studies is continuing, and a series of studies have been completed that has lead to commencement of field vaccination trial of wild 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 built and 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*. In our studies carried out at the BROC, we have shown that the BCG vaccine is protective in badgers by the subcutaneous and mucosal routes, and by the oral route. The problem of the potential destruction of BCG by stomach acids as it passes through the GI tract has been addressed by the development of the lipid-base encapsulation matrix that is able to resist degradation by gastric enzymes (Dr. Frank Aldwell, Otago University, New Zealand).

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.

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 Staten Serum Institute (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: Protective efficacy of BCG vaccination against a low dose challenge with M. bovis

In the wild, under conditions of natural transmission, badgers are exposed to and can become infected with low doses of *M. bovis*. In our initial BROC experimental infection studies we demonstrated that it was possible to infect a badger with

as low <10 colony forming units (CFU) of *M. bovis*. The purpose of the BROC 7 study was to determine what effect vaccination would have on the experimental disease following a low challenge dose and over a longer timescale. Badgers were vaccinated with BCG-Danish strain encapsulated in a semi-solid lipid matrix. This allows for the vaccine to be delivered by the oral route and the matrix protects the vaccine from being degraded in the stomach. A control group remained non-vaccinated. Twelve weeks after vaccination, the badgers were challenged by the endobronchial route with a low dose of *M. bovis* (3×10^2 CFU). At 52 weeks post-challenge the badgers were euthanased and protection assessed by pathology and culture. As measured by distribution and severity of lesions there was significant immune protection generated in the vaccinated group of badgers. The data has given us a much better understanding of how the vaccine is likely to perform under natural conditions. This will facilitate the development of strategies to deliver the vaccine to badger populations.

Delivery of the oral vaccine. *Photography by the Badger Vaccine Project.*

Immunological responses and protective immunity in BCG vaccinated badgers following endobronchial infection with *Mycobacterium bovis*

Lesellier, S.[1], Corner, L.A.L.[1], Costello, E.[2], Lyashchenko, K.[3], Greenwald, R.[3], Esfandiari, J.[3], Singh, M.[4], Hewinson, R.G.[5], Chambers, M.[5], Gormley, E.[1]

[1] UCD TB Diagnostics and Immunology Research Centre, [2] Central Veterinary Research Laboratory, Department of Agriculture, Fisheries and Food,

[3] Chembio Diagnostic Systems, Inc., New York, USA, [4] LIONEX GmbH, Braunschweig, Germany,

[5] Veterinary Laboratories Agency, Weybridge, Surrey, England

European badgers *(Meles meles)* are a reservoir host of *Mycobacterium bovis* and are implicated in the transmission of tuberculosis to cattle in Ireland and Great Britain. The development of a vaccine for use in badgers is considered a key element of any campaign to eradicate the disease in livestock in both countries. In this study we have vaccinated groups of badgers with approximately 5×10^5 cfu of the BCG vaccine delivered via two alternative routes, subcutaneous and mucosal (intranasal/conjunctival). Following experimental endobronchial infection with approximately 10^4 cfu of *M. bovis*, all badgers were euthanised at 12 weeks post-infection. At post-mortem examination both vaccinated groups had significantly reduced severity of disease compared with the non-vaccinated controls. The analysis of immune responses throughout the study showed that vaccination with BCG did not generate any detectable immunological responses as measured by IFN-gamma production in antigen-stimulated peripheral blood mononuclear cells (PBMC) and IgG serological responses. However, the levels of the responses increased following *M. bovis* infection, and the kinetic profiles corresponded to the severity of lesions recorded post-mortem. Significant differences were observed in the timing of development of the immune responses between vaccinates and controls. The results suggest that the immunological responses are associated with the levels of protective immunity and could be used as markers to monitor control of disease in badgers following vaccination.

Immunological responses following experimental endobronchial infection of badgers *(Meles meles)* with different doses of *Mycobacterium bovis*

Lesellier, S.[1], Corner, L.A.L.[1], Costello, E.[2], Sleeman, D.P.[3], Lyashchenko, K.P.[4], Greenwald, R.[4], Esfandiari, J.[4], Hewinson, R.G.[5], Chambers, M.[5], Gormley, E.[1]

[1] UCD TB Diagnostics and Immunology Research Centre, [2] Central Veterinary Research Laboratory, Department of Agriculture, Fisheries and Food,

[3] Department of Zoology, Ecology and Plant Science, University College Cork, [4] Chembio Diagnostic Systems, Inc., New York, USA,

[5] Veterinary Laboratories Agency, Weybridge, Surrey, England

The Eurasian badger *(Meles meles)* is a wildlife reservoir for *Mycobacterium bovis* infection in Ireland and Great Britain and has been implicated in the transmission of tuberculosis to cattle. Vaccination of badgers is an option that could be used as part of a strategy to control the disease. In this study we used an endobronchial infection procedure to inoculate groups of badgers with three different doses (3×10^3, 2×10^2 and <10 Colony Forming Units (CFUs)) of *M. bovis*. After 17 weeks the disease status of each animal was determined by post-mortem pathology and culture for *M. bovis*. Each of the inoculum doses resulted in establishment of infection in the badgers. The cell-mediated immune (CMI) responses were measured by lymphocyte transformation assay (LTA) of peripheral blood mononuclear cells (PBMCs) cultured with

bovine tuberculin (PPD-B). In each infected group the CMI responses increased with a kinetic profile corresponding to the delivered dose and the post-mortem pathology. The serological responses were measured by ELISA and a multi-antigen print immunoassay (MAPIA) in order to investigate any changes in the antigenic repertoire associated with different infective doses. In contrast to the CMI responses, the ELISA and MAPIA showed that the recognition of antigens by the badgers was intermittent and not strongly influenced by the dose of *M. bovis*.

Vaccination of European badgers *(Meles meles)* with BCG by the subcutaneous and mucosal routes induces protective immunity against endobronchial challenge with *Mycobacterium bovis*

Corner, L.A.L.[1], Costello, E.[2], Lesellier, S.[1], O'Meara, D.[2], Gormley, E.[1]

[1] UCD TB Diagnostics and Immunology Research Centre, [2] Central Veterinary Research Laboratory, Department of Agriculture, Fisheries and Food

Tuberculosis 88, 601-609 (2008)

Mycobacterium bovis is endemic in badger *(Meles meles)* populations of Ireland and the United Kingdom and infected badgers are a potential source of infection for cattle. In domestic livestock tuberculosis causes economic losses from lost production and the costs associated with eradication programmes, and in addition there is a risk of zoonotic infection. Whereas culling is currently used to control tuberculous badger populations in Ireland, vaccination, if it were available, would be preferred. A study was undertaken to examine the protective responses of badgers vaccinated either by the subcutaneous or mucosal (intranasal and conjunctival) routes with bacille Calmette-Guérin (BCG), when challenged with *M. bovis* by the endobronchial route. Three groups of badgers were used. The first group (n=4) was vaccinated with approximately 5 x 10^5 colony forming units (cfu) of BCG by subcutaneous injection. In the second group (n=5) badgers were vaccinated via the mucosal route by instilling 1.0 x 10^5 cfu into each conjunctival sac and spraying 1.0 x 10^5 cfu into each nostril (final vaccine dose of 4 x 10^5 cfu). The control (n=5) badgers served as a non-vaccinated group. Twelve weeks post-vaccination all badgers in the three groups were challenged with approximately 10^4 cfu of *M. bovis* by endobronchial inoculation. At 12 weeks post-infection all badgers were examined post-mortem to assess the pathological and bacteriological responses to challenge. Gross and histological lesions of tuberculosis were seen in all challenged badgers and *M. bovis* was recovered from all challenged badgers. However, across six of the eight parameters used to measure disease severity, the infection in the vaccinated badgers was significantly less severe than in the control group. The BCG vaccine induced a significant protective effect in the badgers and the protective immunity was generated by subcutaneous and mucosal vaccination.

Experimental tuberculosis in the European badger *(Meles meles)* after endobronchial inoculation with *Mycobacterium bovis*: II. Progression of infection

Corner, L.A.L.[1], Costello, E.[2], Lesellier, S.[1], O'Meara, D.[1], Gormley, E.[1]

[1] UCD TB Diagnostics and Immunology Research Centre, [2] Central Veterinary Research Laboratory, Department of Agriculture, Fisheries and Food

The aim of the study was to describe, over a period of 24 weeks, the pathological and bacteriological changes in badgers experimentally infected with *Mycobacterium bovis*. The badgers were infected by endobronchial instillation of 2.5 x10^4 colony forming units (cfu) *M. bovis*. After infection, the badgers were examined at 3 weekly intervals when blood and tracheal aspirates were collected. At 6, 12, 18 and 24 weeks post-infection (pi) three animals were euthanized and a detailed pathological and bacteriological examination was performed to assess the nature of the experimental disease. During the course of the study only one badger developed clinical signs of disease: a subcutaneous swelling on its head, first observed at 18 weeks pi. At post-mortem examination gross and histological lesions of tuberculosis were observed and *M. bovis* was recovered from all, except one badger. In the majority of badgers the endobronchial route of inoculation resulted in the establishment of infection that over 24 weeks was non-progressive with limited dissemination of infection from the thoracic cavity, mainly to the hepatic and mesenteric lymph nodes. However, in one of the badgers examined at 18 weeks pi and one at 24 weeks pi, infection was widely disseminated. The disease induced by the endobronchial inoculation displayed the characteristics of disease observed in naturally infected badgers.

Antigen specific immunological responses of badgers *(Meles meles)* experimentally infected with *Mycobacterium bovis*

Lesellier, S.[1, 6], Corner, L.A.L.[1], Costello, E.[2], Sleeman, D.P.[3], Lyashchenko, K.[4], Greenwald, R.[4], Esfandiari, J.[4], Singh, M.[5], Hewinson, R.G.[6], Chambers, M.[6], Gormley, E.[1]

[1] UCD TB Diagnostics and Immunology Research Centre, [2] Central Veterinary Research Laboratory, Department of Agriculture, Fisheries and Food, [3] Department of Zoology, Ecology and Plant Science, University College Cork, [4] Chembio Diagnostic Systems, Inc., New York, USA, [5] Helmholtz Center for Infectious Research; and LIONEX GmbH, Braunschweig, Germany, [6] Veterinary Laboratories Agency, Weybridge, Surrey, England

European badgers *(Meles meles)* are considered to be an important reservoir of infection for *Mycobacterium bovis* and are implicated in the transmission of tuberculosis to cattle in Ireland and Great Britain. 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. Groups of badgers were subjected to necropsy at 6-week intervals and the gross lesion severity status compared with immune responses measured in blood samples taken throughout the course of the study. The panel of antigens included bovine and avian tuberculins (PPD) as well as single antigens, ESAT-6, CFP-10, MPB70, Rv3019c, Rv3873, Rv3878 and Rv3879, all known to be recognised by the immune system in other animal models of tuberculosis infection. Our results demonstrated that *M. bovis* infected badgers responded to specific antigens as early as 6 weeks post-infection, consistent with the presence of visible lesions. The data also revealed unique patterns of antigen recognition with high levels of PBMC proliferation in the presence of CFP-10 but low proliferation levels with ESAT-6. Using a multi-antigen print immunoassay (MAPIA), we were able to confirm that MPB83 is the dominant antigen recognised by serum antibodies in infected badgers.

The vaccine field trial

Whereas captive badger studies are the most cost effective way of examining the protective response to vaccination, such studies cannot be used to predict whether BCG will be protective in free-ranging badgers or to estimate vaccine efficacy. Estimates of vaccine efficacy are extremely valuable in modelling potential vaccine strategies, but data from field trials are needed to reliably estimate protection and vaccine efficacy parameters. Any field trial will by necessity use an oral vaccine delivery system, because this is the likely method of choice for any broad-scale mass vaccination of free-ranging badger populations.

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

In the field trial area in Co. Kilkenny, different proportions (0, 50, and 100%) of the badger population are being vaccinated with BCG or placebo. The advantage of this design is that effects on vaccine efficacy arising from changes in the force of infection as a result of different levels of vaccination coverage can be estimated. The required proportion of vaccinates is achieved by systematically trapping the area. When first encountered, individual badgers are allocated to either the vaccination or control group as required for the particular area. To allow for continued exposure to infection, the trial will be conducted over a 3-yr period. It is estimated that an initial population of 300 badgers (100 in each of the treatment areas) will be required to accurately estimate vaccine efficacy, based on an assumed initial tuberculosis prevalence of 20–30% and vaccine efficacy of 50–70% for an individual badger.

The BCG Danish strain, encapsulated in a lipid formulation for oral administration and containing about 10^8 cfu/ml, is being used in the field trial. Badgers are individually vaccinated by administration of the lipid vaccine or lipid placebo directly into the pharynx. Vaccine and placebo control samples are "double-blind" coded. Badgers will be revaccinated annually and the population examined two times per year by trapping the entire study site in a continuous process. Throughout the trial, estimates of changing tuberculosis incidence will be made from the measurements of humoral immune responses.

At the end of the 3-yr study period, the trial site will be depopulated and all badgers will be examined for tuberculosis by detailed postmortem examination that will include an examination for visible lesions, histologic lesions, and mycobacteriology to demonstrate infection with *M. bovis*. The isolation of *M. bovis* from post-mortem or clinical samples (wound exudates or tracheal swabs) will be used to define a case of tuberculosis. The results and experience gained from the field trial will facilitate the development of strategies for introduction of vaccination into the national program. The trial commenced in 2009.

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

Aznar, I.[1], McGrath, G.[1], Murphy, D.[2], Corner, L.A.L.[2], Gormley, E.[2], Frankena, K.[3], More, S.J.[1], Martin, S.W.[4], O'Keeffe, J.[1, 5], de Jong, M.[3]

[1] UCD CVERA, [2] UCD TB Diagnostics and Immunology Research Centre, [3] Wageningen Institute of Animal Sciences, Wageningen University, The Netherlands, [4] Department of Population Medicine, Ontario Veterinary College, University of Guelph, Ontario, Canada, [5] Department of Agriculture, Fisheries and Food

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

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

Kelly, D.[1], Corner. L.A.L.[2], Gormley, E.[2], Marples, N.M.[1]

[1] School of Natural Sciences, Trinity College Dublin, [2] UCD TB Diagnostics and Immunology Research Centre

This project aims to formulate a protocol for the efficient delivery of BCG vaccine to badgers in order to reduce the prevalence of bovine tuberculosis in wildlife. Working initially with captive animals at the DAFF BROC facility, a number of candidate oral vaccine bait additives were tested as attractants. The captive study demonstrated that carob and cocoa were more attractive to captive badgers than a peanut flavour. The captive animals were required to overturn a floor tile (approximately 2 kg in weight) to gain access to the experimental baits. This proved to be a simple task if they were attracted to the bait. This work will shortly be submitted to the *European Journal of Wildlife Research*.

Some preliminary studies with a wild sett near to the BROC facility showed that the carob powder was a successful attractant for wild badgers. Floor tiles were used to prevent bait access from rodents or birds (as in the captive studies). These preliminary studies also showed that wild animals could learn to remove candidate oral vaccine baits from a simple wax-coated paper package. The ultimate deployment strategy for the oral vaccine bait requires the baits to be packaged. This important finding also informed the design of a wild trial.

In the wild trial, animals were offered a choice of three packaged baits (peanut, carob and cocoa flavours). The baits were buried in 15cm-deep holes adjacent to main sett entrances, and the holes backfilled with earth. The disappearance

of baits from these holes proved that the wild badgers could detect and excavate baits from deep backfilled holes. The results showed that peanut flavour was a more popular attractant than both carob and cocoa butter for wild badgers. No baits were removed from their holes on the first day of presentation, indicating that a pre-baiting period of at least one day would be necessary for live vaccine bait deployment. The wild badgers quickly learned to associate all attractants with the prototype baits and continued to remove them from buried sites, even when the attractants were absent. This suggests that it may be possible to train badgers to feed at a site convenient for the personnel deploying the vaccine baits. A manuscript describing this work is in preparation.

A protocol for a training trial with wild animals, in Wicklow, has been agreed. Fieldwork should be underway by the end of February 2010. It will test whether badgers that are unfamiliar with packaged baits can learn to extract them without additional attractant cues. This may ultimately save money, as the use of attractants on each packaged bait would increase manufacturing costs.

In addition to bait studies, it has been possible to investigate the feeding behaviour of the captive animals at BROC using stable isotope analysis. This has provided a fundamental baseline value for Eurasian badgers which may open the door for more detailed trophic studies both in Ireland and the UK. Additional data collected from animals during their 'winter lethargy' may go some way to understanding how they utilise their stored adipose supplies during the winter months.

Control of TB in wildlife by oral BCG vaccination

Gormley, E.[1], Corner, L.A.L.[1]

[1] UCD TB Diagnostics and Immunology Research Centre

Tuberculosis caused by *Mycobacterium bovis* is present in many wild animal populations throughout the world. The disease can have a significant economic impact when the wild animal species is a reservoir of infection for domestic animals. It can pose a zoonotic threat for those who come in contact with infected animals, be they wild or domesticated. To date, strategies for dealing with TB in wildlife have been limited; physical separation of wild from domestic animals or culling of infected populations can help to reduce the spread of infection, but culling is not an option with species of high conservation value. Vaccination of animals with the bacillus Calmette-Guérin (BCG) vaccine has long been considered an attractive strategy for combating the disease. However, its widespread use in the wild has been constrained pending the development of a suitable delivery system. In the paper under evaluation, Tompkins and colleagues in New Zealand have shown that oral vaccination with BCG can protect wild brushtail possums against natural infection with *M. bovis*. This highly significant finding paves the way towards incorporation of wildlife vaccination into bovine TB eradication programs worldwide.

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Evaluation of badger group territories in four Irish counties by means of colour-marked baits

Smal, C.M.[1], Brown, J.A.[1]

[1] Independent consultants

Badger territorial activity was investigated (in 2002) by a bait-marking technique employed at five study areas located within the Reference Areas of the Four Area Project (Co. Cork, Donegal, Kilkenny and Monaghan). The methodology involved feeding coloured marker pellets, contained within an attractive food mix, to badgers and locating the subsequent dispersal of the defecated pellets. Badgers are social animals that defend their territories both physically and by means of scent marking and defecation sites (such latrine sites are often located along the boundaries of their territories).

The areas selected for study varied in size from c. 5km^2 in Co. Donegal to c. 16km^2 in Co. Kilkenny. 40 setts were fed with marked bait and 34 social groups were identified by evaluation of pellet returns. 1054 latrine sites were identified, with 547 containing marker pellets. The interpreted territory size of the 34 groups varied between 15ha and over 200ha, with a mean of 80ha. Mean badger group density was estimated at 0.8 social groups per km^2. The study area chosen in Co. Donegal was found to possess a very high badger density (c. 1.4 groups per km^2), whereas densities in other counties were closer to expectation.

The badger groups studied displayed relatively well-defined territorial boundaries, typical of those known from other significant studies in Ireland and Britain. There were found examples, in each county, of long-range movements - including movements of individual badgers between main setts and also between territories. Individual badgers may not range over all of the group's territory and it is likely that some badgers are not always present at a group's main sett. These observations have implications for sett survey and also for proposed targeting of badgers with vaccines incorporated into baits.

The study confirmed that each badger group has, almost without exception, one identifiable active main sett within its territory. Field survey of badger setts in any given area may, therefore, be conducted by experienced personnel with enumeration of active main setts and with expectation of reliable assessment of badger group density.

Tuberculosis in European badgers *(Meles meles)* and the control of infection with bacillus Calmette-Guérin vaccination

Corner, L.A.L.[1], Murphy, D.[1], Costello, E.[2], Gormley, E.[1]

[1] UCD TB Diagnostics and Immunology Research Centre, [2] Central Veterinary Research Laboratory, Department of Agriculture, Fisheries and Food

The eradication of tuberculosis (*Mycobacterium bovis* infection) from cattle herds may be compromised if infected wildlife species, such as European badgers *(Meles meles)*, share the same environment and contribute to transfer of infection. Options for dealing with tuberculosis in this wild reservoir host are limited by conservation and social concerns, despite a clear implication that infected badgers are involved with the initiation of tuberculosis in cattle herds. Vaccination of badgers against *M. bovis*, if successfully employed, would directly facilitate the completion of bovine tuberculosis eradication in affected areas. Vaccine trials in captive badgers have established that the *M. bovis* bacille Calmette-Guérin (BCG) vaccine can induce a protective response that limits the distribution and severity of tuberculosis disease following experimental challenge. The protective effect of the vaccine has been demonstrated when the vaccine was delivered by subcutaneous injection, deposited on mucous membranes, and given orally in a lipid formulation. A large-scale field trial of oral BCG vaccine has been designed to measure the protection generated in wild badgers subjected to natural trans-

Supporting studies

Badger immunodiagnostics

Development of badger immunodiagnostics

In tandem with the badger vaccine research, we have been developing and assessing a range of immunodiagnostic tests that will be required for tuberculosis surveillance in badger populations to monitor the effect of vaccination. This work has been carried out in collaboration with VLA Weybridge. More recently we have been working with Enfer group on developing antibody based diagnostic tests for badgers. This has the advantage of using serum samples, which can be easily stored prior to testing and removes the impediment for immediate transport of blood samples to the laboratory for testing.

Sensitivity of immunological assays for diagnosis of tuberculosis in wild badgers

A range of serological and cell mediated immunity (CMI) based assays have been developed recently and have shown encouraging results when used in captive badgers. However, there is a need to validate these tests on naturally infected badgers. The aim of the current study was to evaluate five of these diagnostic immunoassays (the Brock test, the BrockTB STAT-PAK®, the lymphocyte transformation assay (LTA), the interferon-gamma (IFN-γ) ELISA and the ELISPOT assay) in wild badgers by comparing test results to the diagnostic gold standard, i.e. bacterial culture using a comprehensive range of lymph nodes and tissues collected from badgers. Blood samples were collected from culled badgers (n = 215) prior to euthanasia and all badgers were examined *post mortem* with bacterial culture of tissues. Based on ROC analysis, the CMI assays had higher sensitivities than the serological assays in this study, the LTA having the highest sensitivity (54.9%, apparent specificity 86.6%) and the BrockTB STATPAK the lowest sensitivity (30.8%, apparent specificity 97.8%). The sensitivity of all the immunodiagnostic assays improved as the disease severity increased and this was more pronounced with the serological based assays than the CMI assays. Overall, the individual immunodiagnostic assays assessed in this study had low sensitivities for the accurate diagnosis of *M. bovis* infection in badgers when compared to the gold standard of bacteriological culture. However, the combination of two or more assays improved the diagnostic accuracy of the tests.

Adverse reactions to vaccine usage

Adverse reactions to *Mycobacterium bovis* bacille Calmette–Guérin (BCG) vaccination against tuberculosis in humans, veterinary animals and wildlife species

Murphy, D.[1], Corner, L.A.L.[1], Gormley, E.[1]

[1] UCD TB Diagnostics and Immunology Research Centre

The *Mycobacterium 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. Despite the large number of doses delivered since its first usage in 1921, reports of adverse reactions arising from the use of the BCG vaccine are relatively uncommon and where serious reactions do occur they are often the result of vaccination of immuno-compromised individuals. Factors that may influence the development of adverse reactions to BCG include the potency and dose of the vaccine strain, the route of delivery, the age and immune status of the host, and the skill levels of the operator administering the vaccine. Circumstances affecting the notification of adverse reactions include the lack of clear case definitions of abnormal vaccine reactions, and a scarcity of systematic surveillance and functioning reporting systems. With continued use of the BCG and the development of a new generation of prophylactic and therapeutic vaccines against tuberculosis in different host species, the risk factors associated with adverse reactions may need to be reappraised.

Mathematical modelling

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

Aznar, I.[1], McGrath, G.[1], Corner, L.A.L.[2], Gormley, E.[2], Frankena, K.[3], More, S.J.[1], Martin, S.W.[4], O'Keeffe, J.[1, 5], de Jong, M.[3]

[1] UCD CVERA, [2] UCD TB Diagnostics and Immunology Research Centre, [3] Wageningen Institute of Animal Sciences, Wageningen University, The Netherlands, [4] Department of Population Medicine, Ontario Veterinary College, University of Guelph, Ontario, Canada, [5] Department of Agriculture, Fisheries and Food

Recent research indicates that bovine tuberculosis (bTB, infection with *Mycobacterium bovis*) is endemic in the badger population in Ireland, and that disease in badgers contributes to the problem in cattle. Despite efforts to reduce cattle-to-cattle transmission through a comprehensive national surveillance program and the reactive badger culling policy, bTB has remained at a relatively constant, albeit low, prevalence in the cattle population of Ireland. It is thought that a strategy involving vaccination of badgers, possibly in combination with other intervention strategies, will help to reduce the incidence of infection in badgers. Modelling various scenarios involving a number of different interventions will help determine the impact that these interventions may have on the bTB prevalence in both cattle and badger, and will help to inform further research and policy decisions. The main objective is to assess the impact of interventions on bovine tuberculosis (bTB) prevalence in cattle and badgers; for this a mathematical model of bTB transmission that describes the disease in cattle and badgers in the Republic of Ireland is being developed. A conceptual framework of how bTB persists in both badgers and cattle has been constructed, using mathematical modelling. The next step of the project will

involve the use of existing data to get quantitative estimates of the required model parameters. Irish data on cattle to cattle transmission have been analysed and the transmission parameter for cattle (β) has been estimated. The estimation of the inter-species transmission parameter is in progress.

Badger anaesthesia, clinical pathology

Bronchoalveolar lavage cytology from captive badgers

McCarthy, G.[1], Shiel, R.[1], O'Rourke, L.[1], Murphy, D.[2], Corner, L.A.L.[2], Costello, E.[3], Gormley, E.[2]

[1] UCD School of Agriculture, Food Science and Veterinary Medicine, [2] UCD TB Diagnostics and Immunology Research Centre,

[3] Central Veterinary Research Laboratory, Department of Agriculture, Fisheries and Food

Bronchoalveolar lavage (BAL) fluid is evaluated for the diagnosis and study of lung disease and airway inflammation. Cytologic profiles for BAL fluid have not been reported for badgers and may be useful in understanding the pathogenesis of pulmonary diseases such as *Mycobacterium bovis*. The aim of this study was to evaluate cytologic and microbial findings in BAL fluid from captive European badgers *(Meles meles)* and identify correlates with the results of concurrently collected blood and fecal samples. BAL fluid (by a nonbronchoscopic method) and jugular venous blood samples (for routine CBC) were obtained from 23 captive tuberculosis- free anesthetized badgers on 2 occasions 4 weeks apart. Fecal samples were collected for routine parasitology. Morphologic evaluation and 100- cell differentials were done on cytocentrifuged BAL specimens. Pellets from centrifuged BAL were aerobically cultured for bacteria. With the 2 BAL samples from each of the 23 badgers combined, the median (range) cell percentages were 73.0% (5–95%) neutrophils, 7.5% (2–16%) macrophages, 8.0% (0–27%) lymphocytes, and 9.5% (0–92%) eosinophils. Macrophages frequently contained silica-like crystals. Other findings included ciliated epithelial cells, goblet cells, mucus, and *Aelurostrongylus* sp. larvae. A light growth of *Streptococcus*, *Pasteurella*, or *Escherichia coli* was cultured in 6 badgers. *Trypanosoma pestanai* were identified in blood from 10 badgers and fecal parasites (mainly coccidia) were found in 20 badgers. No correlation was found between BAL and CBC results and the presence of parasites. The predominance of neutrophils in BAL fluid from badgers differs from the predominance of macrophages found in BAL from other species. This difference may reflect the burrowing lifestyle or the unique immune response of badgers.

The national programme

A review of TB research in Ireland

More, S.J.[1], Gormley, E.[2]

[1] UCD CVERA, [2] UCD TB Diagnostics and Immunology Research Centre

Research on bovine tuberculosis (TB) has been conducted in Ireland over the last 20 years or so. Much of this work has focused on gaps in knowledge of disease epidemiology, a critical assessment of disease control strategies, constraints to eradication and an evaluation of alternative strategy options. As yet, no summary is available of this substantial body of peer-reviewed work. This review paper seeks to fill this gap.

What is needed to eradicate bovine tuberculosis successfully: an Irish perspective

More. S.J.[1]

[1] UCD CVERA

The Veterinary Journal 180, 275-278 (2009)

The Irish national bovine tuberculosis (TB) eradication programme, first established in 1954, has proved effective in controlling TB caused by *Mycobacterium bovis*. However, eradication of the disease remains elusive. We now have a good understanding of the constraints to eradication, based on detailed research conducted in Ireland and elsewhere. But three critical issues will need to be resolved if Ireland is to be successful in moving from TB control to eradication:

- methods to sustainably prevent wildlife-to-cattle transmission;
- the introduction of measures for improved cattle controls, and
- a critical rethink of programme governance.

Any impact from the latter two requirements is likely to be severely limited prior to the introduction of the first, given that badgers can be considered an 'upstream' driver, with ongoing transmission of infection from badgers to cattle irrespective of current efforts to minimise cattle-to-cattle transmission. Each of these three issues are discussed in some detail in this 'Personal View' to *The Veterinary Journal*.

Quality control

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

Good, M.[1], Clegg, T.A.[2], Murphy, F.[1], More, S.J.[2]

[1] Department of Agriculture, Fisheries and Food, [2] UCD CVERA

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

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

Good, M.[1], Clegg, T.A.[2], Costello, E.[3], More S.J.[4]

[1] Department of Agriculture, Fisheries and Food, [2] UCD CVERA, [3] Central Veterinary Research Laboratory, Department of Agriculture, Fisheries and Food

Ireland currently obtains its avian and bovine tuberculin purified protein derivatives (PPDs) from a single source. Problems of supply or quality cannot be discounted. As yet, however, no work has been reported on the impact of tuberculins of different potencies on the performance of the single intradermal test (SIT) and the single intradermal comparative tuberculin test (SICTT). Therefore, the aim of this study was to compare the performance of bovine tuberculin PPDs of different potencies on the performance of the SICTT and SIT, as currently performed in Ireland and throughout Europe. The study was randomised, controlled and blinded. Three trial bovine tuberculins of varying potency, as assayed in naturally infected bovines, (low [1,192 IU/dose], normal [6,184], high [12,554]) were used in this study. Three SICTTs (based on the three trial tuberculins) were conducted on 2,102 animals. The results of these tests were compared, based on reactor-status and skin increases at the bovine site. There was a significant difference in the number of reactors detected using the high and low potency tuberculin. When used in a SICTT, the high potency and low potency tuberculins detected 40% more and 50% less reactors, respectively, than normal potency tuberculin. Further, the low potency tuberculin [X] failed to detect 20% of 35 animals with visible lesions. Tuberculin of different potencies will impact on reactor disclosure rates.

The development and application of new measures of performance

Higgins, I.[1], Williams, D.[2], More, S.J.[1]

[1] UCD CVERA, [2] UCD School of Mathematical Sciences

In recent years, there have been substantial advances in the analysis and management of the national bovine tuberculosis database in Ireland. The national Animal Health Computer System was upgraded in 2005, and facilitates the central collection and management of national data. This now provides over twenty years of data for research purposes. Furthermore, there has 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. With these advances, new statistical measures have been developed to assist with decision-making, both locally and at a national level. These measures, which relate to both programme activity and performance, are consistent with recommendations from several recent international meetings, including a shift towards herd-based measures, and clear differentiation between activities (and outcomes) relating to surveillance and to control. These measures will be incorporated within the national reporting system, to provide decision-makers with timely and objective information relevant to programme management, and further progress towards bovine tuberculosis control and eradication. The objectives of this study were to define performance measures to assist with ongoing programme review, create automated means to enable ongoing measurement, and to create an episode based summary file of bovine tuberculosis (TB) restrictions for the period 1989-2009. Progress has been made in correctly identifying, classifying and summarising periods of possible *Mycobacterium bovis* infection as indicated by TB test result data. Measures based on restriction based episodes have been defined and suggestions made as to how these can be summarised in a way that provides valid identification of trends and comparisons between regions.

Public health

An outbreak of tuberculosis affecting cattle and people on an Irish dairy farm, following the consumption of raw milk

Doran, P.[1], Carson, J.[2], Costello, E.[3], More, S.J.[4]

[1] Department of Agriculture, Fisheries and Food, [2] Wexford General Hospital,

[3] Central Veterinary Research Laboratory, Department of Agriculture, Fisheries and Food, [4] UCD CVERA

Bovine tuberculosis is an ongoing problem in Ireland, and herd incidence has remained at approximately 5% for some years. Spillover of infection from cattle to people remains an ever-present possibility, given the ongoing pool of infection in the Irish cattle population. This paper describes an outbreak of tuberculosis affecting cattle and people on a dairy farm in southeastern Ireland following the consumption of milk from a seven-year-old cow with tuberculous mastitis. Twenty-five of 28 calves born during autumn 2004 and spring 2005 were subsequently identified as TB reactors, and five of six family members were positive on the Mantoux test. During 2005, milk from this cow had mainly been used to feed calves, and was added only occasionally to the bulk tank. Therefore, the calves each received infected milk on an almost continuous basis between birth and weaning. The family collected milk from the bulk milk tank, and consumed it without pasteurisation. This case highlights the risks associated with the consumption of raw milk. In this family, TB has had a very significant impact on the health of two young children. These risks are well recognised, and relevant information for farmers is available. It is of concern, therefore, that raw milk consumption remains prevalent on Irish farms. New strategies are needed, in partnership with industry, to address this important issue.

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North-South linkages

An all-island approach to mapping bovine tuberculosis in Ireland

McGrath, G.[1], Abernethy, D.[2], Stringer, L.[2, 3], More, S.J.[1]

[1] UCD CVERA, [2] Department of Agriculture and Rural Development, Belfast, Northern Ireland,

[3] Institute of Veterinary, Animal and Biomedical Sciences, Massey University, Palmerston North, New Zealand

This study used techniques in Geographical Information Systems (GIS) to explore the spatial patterns of bovine tuberculosis (TB) in the whole island of Ireland over an 11-year period. This is the first time that data pertaining to TB from the Republic of Ireland and Northern Ireland have been collated and examined in an all-Ireland context. The analyses were based on 198,156 point locations representing active farms with cattle in Northern Ireland and the Republic of Ireland between the years 1996 and 2006. The results consist of a series of maps giving a visual representation of cattle populations and associated detected bTB levels on the island of Ireland over this time interval.

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Bovine tuberculosis trends in the United Kingdom and Ireland, 1995 to 2008

Downs, S.[1], Broughan, J.[1], Clifton-Hadley, R.[1], Goodchild, T.[1], Upton, P.[1], de la Rua-Domenech, R.[2], Rolfe, S.[3], Blissett, M.[4], Abernethy, D.[5], Menzies, F.[5], Clark, N.[5], More, S.J.[6], Higgins, I.[6], McGrath, G.[6], Duignan, A.[6, 7]

[1] Veterinary Laboratories Agency, Weybridge, Surrey, England, [2] Department of Environment, Food and Rural Affairs, United Kingdom,

[3] Welsh Assembly Government, Cardiff, Wales, [4] Scottish Government, Edinburgh, Scotland, [5] Department of Agriculture and Rural Development, Belfast, Northern Ireland, [6] UCD CVERA, [7] Department of Agriculture, Fisheries and Food

Different strategies in national bovine tuberculosis (BTB) control programmes are employed in Great Britain (namely England, Scotland and Wales), Northern Ireland and the Republic of Ireland, reflecting differences in perceived risk factors and programme priorities. Comparing the effects of these strategies or underlying BTB trends between the jurisdictions is hampered by different case definitions and test parameters used within each jurisdiction. This project is being conducted to address these concerns, with the aim to describe long-term BTB trends in the UK and Ireland during 1995 to 2008.

Density of TB incidence

Legend:
- < 0.005
- 0.005 - 0.009
- 0.01 - 0.014
- 0.015 - 0.019
- 0.02 - 0.024
- 0.025 - 0.029
- 0.03 - 0.034
- 0.035 - 0.039
- 0.04+
- Non-agricultural Land
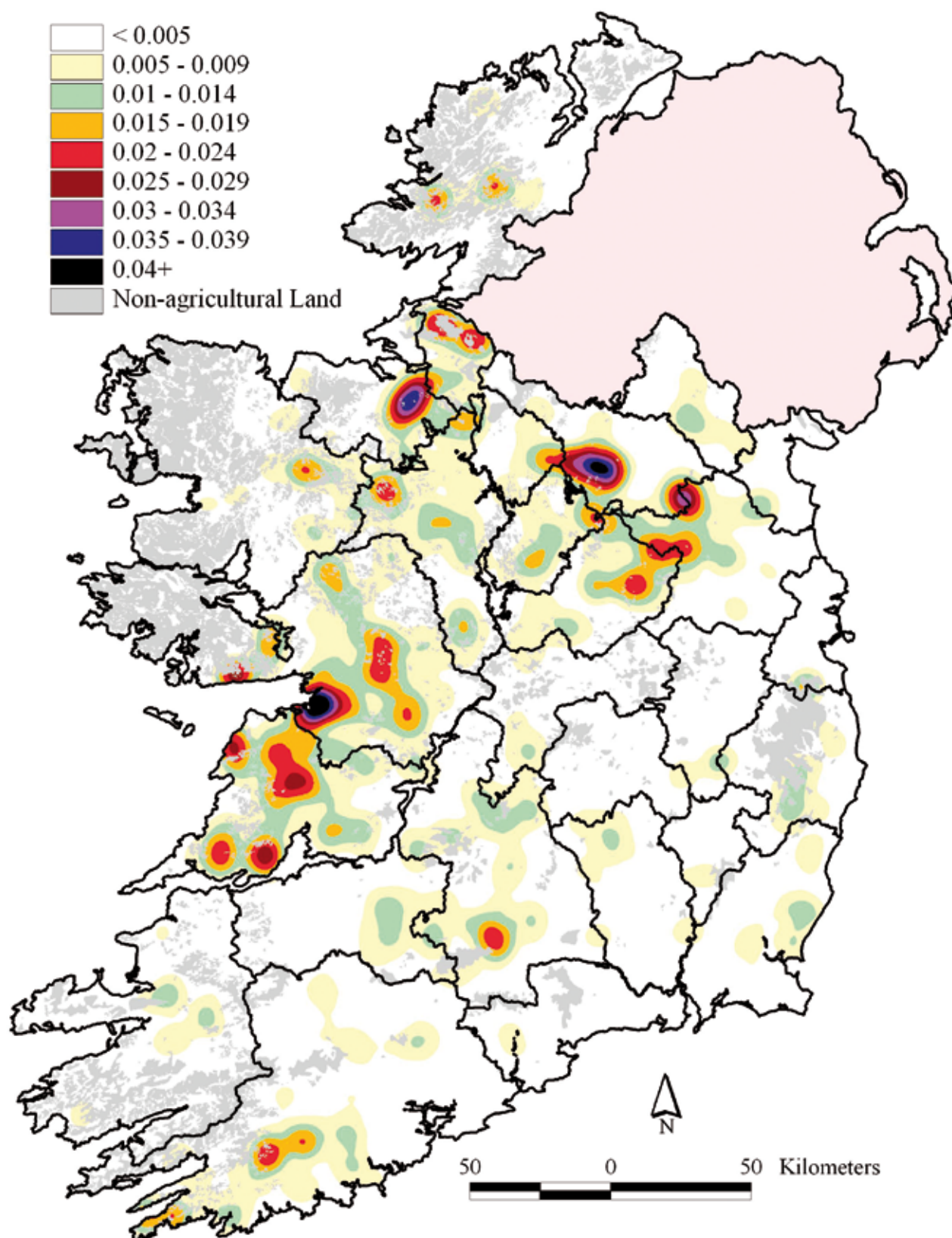
Density of TB incidence per square km during 2008 (kernel density with search radius at 10km).
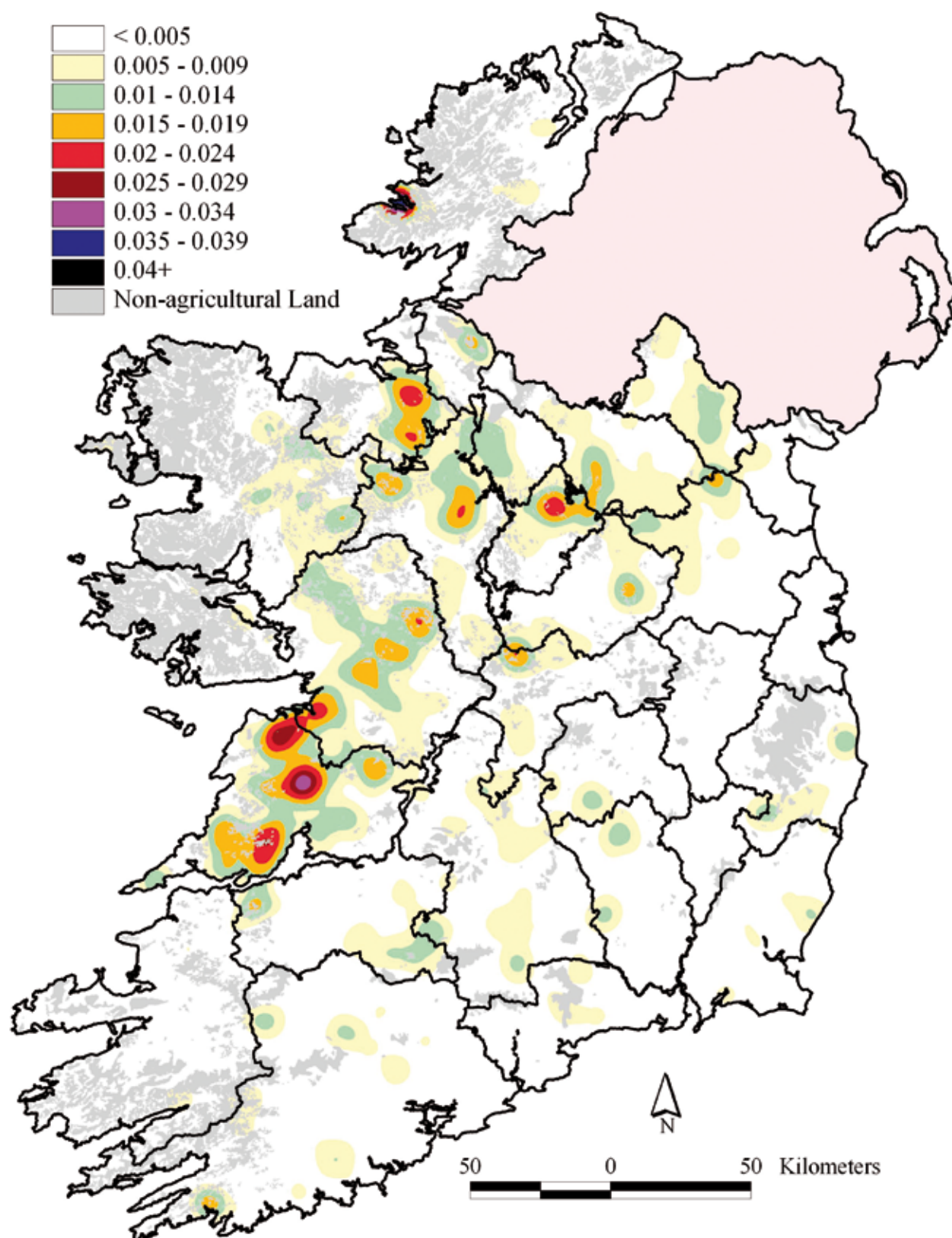
Density of TB incidence per square km during 2009 (kernel density with search radius at 10km).

APT per DED
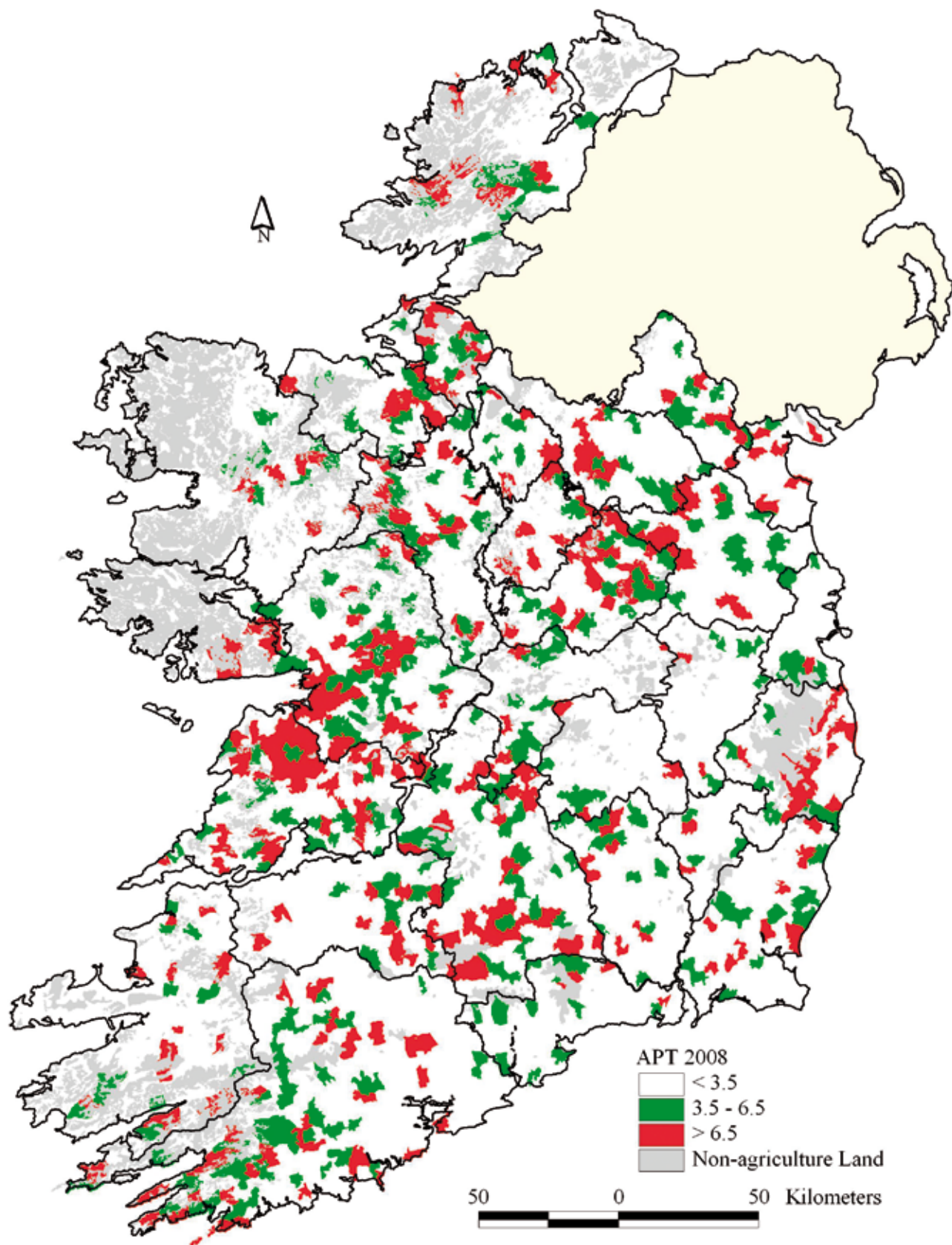
APT 2008

	< 3.5
	3.5 - 6.5
	> 6.5
	Non-agriculture Land

50   0   50 Kilometers

APT (reactors per 1000 tests) per district electoral division, 2008.

APT 2009

	< 3.5
(green)	3.5 - 6.5
(red)	> 6.5
(grey)	Non-agriculture Land

50   0   50   Kilometers
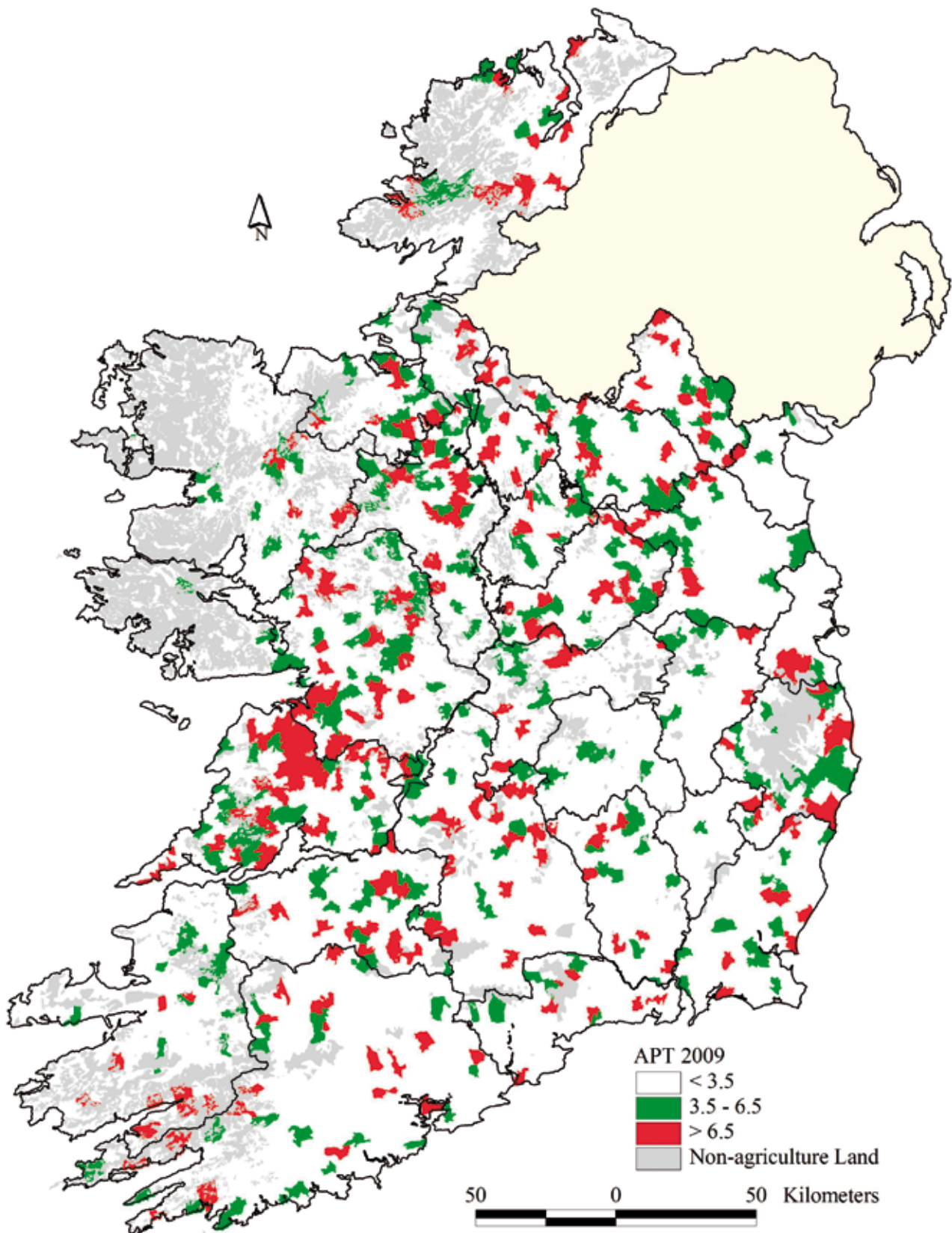
APT (reactors per 1000 tests) per district electoral division, 2009.

Herd health

Animal Health Ireland

Herd health prompts concern over international competitiveness

More, S.J.[1]

[1] UCD CVERA

Irish Farmers Journal (Animal Health Supplement), 15 March 2008

Health problems adversely affect both on-farm profitability and international competitiveness. Farmers with high SCC problems can lose €10-40K each year, primarily through reduced production and increased culling costs. The UCD Herd Health group recently calculated losses of €140K/year on one farm in Leinster. However, international competitiveness is the greater concern, as it affects on-farm profitability in the longer-term. International competitiveness is affected by both standards (what farmers are required to do) and quality (what farmers achieve). In the area of animal health, standards in Ireland are high, but quality is variable. Quality is a relative term, and Ireland's performance must, and will, be judged against the performance of key competing nations. A range of issues have been addressed (or are now being tackled) by key competitors, including mastitis, fertility, lameness, Johne's disease, IBR, BVD etc.

What is herd health? It concerns the development and implementation of national and on-farm strategies to address specific herd health challenges; those issues that are likely to impact on on-farm profitability and international competitiveness in 5 to 10 years time. The critical ingredients to success include long-term planning, commitment, national cooperation, long-term funding and the best-available technical advice. It is much more than just laboratory testing and vaccination, although these may play a role in some issues.

Health problems (for example, mastitis, fertility, IBR, BVD, Johne's disease) are issues for industry (farmers, processors), not government. In a modern society, the role of government is to support public goods and services (hospitals, schools, the gardai, public transport links etc), which are institutions available to all and funded through compulsory taxation. The benefits from improved herd health will mainly flow to industry rather than the general public.

Herd health is being proactively addressed in a number of countries (particularly in northern Europe and Australasia), with important lessons for Ireland. These countries each have whole-of-sector organisations (such as the Swedish Dairy Industry, the Dutch Dairy Board, Dairying Australia, Meat and Livestock Australia) with broad-ranging national responsibility for national development, policy, animal health, national and international marketing, research etc. Each of these organisations is run and funded by industry, generally through an annual levy on farmers and processors. Government's role (including funding) in these sectors is very limited; under international law, they are obliged to play a regulatory role, and in several countries also contribute in the area of research. These organisations are leading international efforts in innovative, successful herd health programmes. For example, Sweden and Denmark have virtually eradicated BVD, with Switzerland now following suit. The Danes, Dutch and Australians have each been tackling Johne's disease (JD) for the last 10-15 years, and from 2011 the Dutch Dairy Board will no longer pick up milk from high-risk JD farms. Costing the industry approximately €10 million in 2008, this strategy is being taken to enhance the international competitiveness of Dutch dairy product into the future, particularly with infant formula. Dairy Australia has supported the development and operation of national programmes to address mastitis (Countdown Downunder) and fertility (InCalf) concerns, with considerable success.

Ireland cannot keep up with international progress in product quality, unless it moves to address a range of health concerns. There are a broad range of issues to address, including the fragmented nature of industry, long-term industry funding, structures to support long-term planning and implementation, etc. Over the last four years, there have been discussions about the establishment of a national herd health initiative, but progress has been slow. We need to act soon, to maximise both the standards and quality of livestock products from Irish farms into the future.

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A case for increased private sector involvement in Ireland's national animal health services

More, S.J.[1]

[1] UCD CVERA

Non-regulatory animal health issues, such as Johne's disease, infectious bovine rhinotracheitis (IBR) and mastitis will become increasing important, with ongoing globalisation of markets in animals and animal products. In response, Ireland may need to broaden the scope of its national animal health services. However, there have been concerns about the respective roles and responsibilities (both financial and otherwise) of government and industry in any such moves. This paper argues the case for increased private sector involvement in Ireland's national animal health services, based both on theoretical considerations and country case studies (the Netherlands and Australia). The Dutch and Australian case studies present examples of successful partnerships between government and industry, including systems and processes to address non-regulatory animal health issues. In each case, the roles and responsibilities of government are clear, as are the principles underpinning government involvement. Furthermore, the roles and responsibilities (financial and otherwise) of the Dutch and Australian industry are determined through enabling legislation, providing both legitimacy and accountability. There are constraints on the use of EU and national government funds to support non-regulatory animal health services in EU member states (such as Ireland and the Netherlands).

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Setting priorities for non-regulatory animal health in Ireland: results from an expert Delphi Study and a farmer priority identification survey

More, S.J.[1], McKenzie, K.[2], O'Flaherty, J.[3], Doherty, M.L.[4], Cromie, A.R.[5], Magan, M.J.[3]

[1] UCD CVERA, [2] UCD Geary Institute, [3] Animal Health Ireland, [4] UCD School of Agriculture, Food Science and Veterinary Medicine,

[5] Irish Cattle Breeding Federation Society Ltd.

Agriculture is a very important contributor to the Irish economy. In Ireland, national animal health services have been a government, rather than an industry, responsibility. In 2009, Animal Health Ireland (AHI) was established to provide a partnership approach to national leadership of non-regulatory animal health issues (those not subject to national and/or EU regulation). The objectives of this study were to elicit opinion from experts and farmers about non-regulatory animal health issues facing Irish livestock industries, including prioritisation of animal health issues and identification of opportunities to maximise the effective use of AHI resources. The study was conducted with experts using Delphi methodology over 3 rounds, and with farmers using a priority identification survey. Non-regulatory bovine diseases/conditions were prioritised by both experts and farmers based on impact and international competitiveness. For each high priority diseases/condition, experts were asked to provide an assessment based on cost, impact, international perception, impediment to international market access and current resource usage effectiveness. Further information was also sought from experts about resource allocation preferences, methods to improve education and coordination, and innovative measures to improve prevention and management. There was close agreement between responses from experts and dairy farmers: each gave highest priority to 3 biosecure (bovine viral diarrhoea [BVD], infectious bovine rhinotracheitis [IBR], paratuberculosis) and 4 non-biosecure (fertility, udder health/milk quality, lameness, calf health) diseases/conditions.

Beef farmers also prioritised parasitic conditions and weanling pneumonia. The adverse impact of biosecure diseases is currently considered relatively minor by experts, but would increase substantially in time. There are already substantial costs to farms and agribusiness from non-biosecure diseases/conditions. Experts preferred an equal allocation of resources between these biosecure and non-biosecure diseases/conditions, with emphasis on adopting/adapting international models, education and awareness-raising. The results from this study provide robust insights about non-regulatory animal health priorities in Ireland, as perceived by experts and farmers, using methodologies that are both transparent and inclusive. They have already been extremely influential in shaping national policy, as a foundation for interdisciplinary (and multi-agency) cooperation, as a contribution to efforts to encourage stakeholder responsibility-taking, and to ongoing development of postgraduate and undergraduate veterinary education in Ireland.

Animal Health Ireland: shaping the future of non-regulatory animal health in Ireland through national coordination and partnership

More, S.J.[1], O'Flaherty, J.[2], Doherty, M.L.[3], Downey, L.[3], McKenzie, K.[4], Magan, M.J.[2]

[1] UCD CVERA, [2] Animal Health Ireland, [3] UCD School of Agriculture, Food Science and Veterinary Medicine, [4] UCD Geary Institute

Ireland has made substantial progress in regulatory animal disease control, through a range of government-led national programmes. In contrast, progress on a range of non-regulatory animal health issues have suffered from an absence of national leadership and management. This paper describes the establishment and early activities of Animal Health Ireland, a recently-established organisation that seeks to shape the future of non-regulatory animal health in Ireland through national co-ordination and partnership.

Food chain control – pre-harvest phase

More, S.J.[1]

[1] UCD CVERA

A global approach is needed, from farm to table, to ensure that food for humans is safe to eat. Controls at the first stage of the food chain, the pre-harvest phase, are a critical part of this broader approach. In this paper, attention is paid to three broad components of food chain control at this stage, including the safety of animal feeds, animal health, and traceability. With each of these components, a range of examples are used to highlight key points.

Fertility

Oestrous synchronisation in cattle – current options following the EU regulations restricting use of oestrogenic compounds in food-producing animals: a review

Lane, E.A.[1], Austin, E.J.[2], Crowe, M.A.[2]

[1] UCD CVERA, [2] UCD School of Agriculture, Food Science and Veterinary Medicine

Oestrous synchronisation is an important strategy to improve reproductive management of cattle. The use of oestradiol-17β, and its related ester derivatives, in food-producing cattle for the purposes of oestrous synchronisation is prohibited in the European Union since October 2006; a serious limitation in the implementation of large-scale use of cost effective synchronisation regimens in both dairy and beef herds. This has obvious consequences within the EU and also in other countries that have restricted the use of oestradiol following the EU ban. Oestrous synchronisation is an important facilitator for the use of artificial insemination, a necessary part of any national herd genetic improvement scheme. Presently, only 35% of the Irish dairy herd is bred by artificial insemination; and facilitation rather than restriction is required to increase this percentage. Ideally synchronisation of oestrus should increase submission rates, improve or at least not affect conception rates, and thus, increase overall pregnancy rate at the end of the breeding season. This should reduce the proportion of cows to be culled. This paper aims to review the oestrous synchrony options available in EU countries and other countries affected by the European ban on oestrogenic compounds being used for oestrous synchrony protocols. Currently, the options available for oestrous synchronisation are generally not as effective, efficient or cost effective as those that incorporated use of oestrogenic compounds.

Key factors affecting dairy cow fertility in Ireland

Lane, E. A.[1], Crowe, M.A.[2], Wickham, B.[3], More, S.J.[1]

[1] UCD CVERA, [2] UCD School of Agriculture, Food Science and Veterinary Medicine, [3] Irish Cattle Breeding Federation Society Ltd.

Reproductive targets must be achieved to ensure long-term economic viability of intensively managed dairy herds. Many studies have highlighted the costs associated with poor fertility. Herd management can be effectively facilitated by use of integrated computerised programmes for fertility, health and production. Large and expanding herds may certainly benefit from use of these computerized packages. The analysis of herd management records allows for accurate assessment of the current status of the herd, a crucial decision making tool to implement effective change. Monitoring of such changes to ensure their effectiveness is essential to the success of any programme, while, participation in discussion groups, allows for peer comparisons, a key factor in motivating herd management change. This programme of work aims to i) evaluate the reproductive efficiency of the national dairy herd utilizing the Irish Cattle Breeding Federation's (ICBF) database, ii) determine the key drivers of lowered fertility in the national herd, iii) correlate production and management strategies with reproductive performance, and iv) monitor the impact of changes in herd management on the reproductive performance of Irish dairy herds.

Milk quality

Global trends in milk quality: implications for the Irish dairy industry

More, S.J.[1]

[1] UCD CVERA

The quality of Irish agricultural product will become increasingly important with the ongoing liberalisation of international trade. This paper presents a review of the global and Irish dairy industries; considers the impact of milk quality on farm profitability, food processing and human health; examines global trends in quality; and explores several models that are successfully being used to tackle milk quality concerns. There is a growing global demand for dairy products, fuelled in part by growing consumer wealth in developing countries. Global dairy trade represents only 6.2% of global production and demand currently outstrips supply. Although the Irish dairy industry is small by global standards, approximately 85% of annual production is exported annually. It is also the world's largest producer of powdered infant formula. Milk quality has an impact on human health, milk processing and on-farm profitability. Somatic cell count (SCC) is a key measure of milk quality, with a SCC not exceeding 400,000 cells/ml (the EU milk quality standard) generally accepted as the international export standard. There have been ongoing improvements in milk quality among both established and emerging international suppliers. A number of countries have developed successful industry-led models to tackle milk quality concerns. Based on international experiences, it is likely that problems with effective translation of knowledge to practice, rather than incomplete knowledge per se, are the more important constraints to national progress towards improved milk quality.

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Milk quality in Ireland during 2003 to 2007: a critical review of national policy and performance

More, S.J.[1], Clegg, T.A.[1], O'Grady, L.[2]

[1] UCD CVERA, [2] UCD School of Agriculture, Food Science and Veterinary Medicine

Ireland is an important player in the world dairy trade and the world's leading producer of infant nutrition products. This paper presents an overview of national milk policy and of objective measures of milk quality in Ireland during 2003 to 2007. Relevant legislation and legislative enforcement are reviewed. An analysis was undertaken of somatic cell count (SCC) results from all milk-recorded herds in Ireland during 2003 to 2007. Additional data were obtained from unpublished government reports. A high percentage of herds have high SCC results, with results fluctuating seasonally. The percentage of herds defined as 'problematic' is heavily influenced by legislative interpretation. There are a range of factors to consider when addressing these concerns including an increased focus on competitive advantage, a shift in focus from input- to output-based standards, and review of current approaches to national governance of milk quality issues.

Farm management factors associated with bulk tank total bacterial count in Irish dairy herds during 2006/2007

Kelly, P.T.[1,2], O'Sullivan, K.[3], Berry, D.P.[1], More, S.J.[4], Meaney, W.J.[1], O'Callaghan, E.J.[1], O'Brien, B.[1]

[1] Teagasc Moorepark Dairy Production Research Centre, [2] UCD School of Agriculture, Food Science and Veterinary Medicine,

[3] UCC Department of Statistics, [4] UCD CVERA

Research has shown that total bacterial count (TBC), which is the bacterial growth per ml of milk over a fixed period of time, can be decreased by good hygiene and farm management practices. The objective of the current study was to quantify the associations between herd management factors and bulk tank TBC in Irish spring calving, grass-based dairy herds. The relationship between bulk tank TBC and farm management and infrastructure was examined using data from 400 randomly selected Irish dairy farms where the basal diet was grazed grass. Herd management factors associated with bulk tank TBC were identified using linear models with herd annual total bacterial score (i.e., arithmetic mean of the natural logarithm of bulk tank TBC) included as the dependent variable. All herd management factors were individually analysed in a separate regression model that included an adjustment for geographical location of the farm. A multiple stepwise regression model was subsequently developed. Median bulk tank TBC for the sample herds was 18,483 cells/ml ranging from 10,441 to 130,458 cells/ml. Results from the multivariate analysis indicated that the following management practices were associated with low TBC; use of heated water in the milking parlour; participation in a milk recording scheme; and tail clipping of cows at a frequency greater than once per year. Increased level of hygiene of the parlour and cubicles were also associated with lower TBC. Herd management factors associated with bulk tank TBC in Irish grazing herds were generally in agreement with most previous studies from confinement systems of milk production.

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Farm management factors associated with bulk tank somatic cell count in Irish dairy herds

Kelly, P.T.[1,2], O'Sullivan, K.[3], Berry. D.P.[1], More, S.J.[4], Meaney, W.J.[1], O'Callaghan, E.J.[1], O'Brien, B.[1]

[1] Teagasc Moorepark Dairy Production Research Centre, [2] UCD School of Agriculture, Food Science and Veterinary Medicine,

[3] UCC Department of Statistics, [4] UCD CVERA

The relationship between bulk tank somatic cell count (SCC) and farm management and infrastructure was examined using data from 398 randomly selected, yet representative, Irish dairy farms where the basal diet is grazed grass. Median bulk tank SCC for the farms was 282,887 cells/ml ranging from 82,209 to 773,028 cells/ml. Two questionnaires were administered through face-to-face contact with each farmer. Herd-level factors associated with bulk tank SCC were determined using linear models with annual somatic cell score (i.e., arithmetic mean of the natural logarithm of bulk tank SCC) included as the dependent variable. All herd level factors were analysed individually in separate regression models, which included an adjustment for geographical location of the farm; a multiple regression model was subsequently developed. Management practices associated with low SCC included the use of dry cow therapy, participation in a milk recording scheme and the use of teat disinfection post-milking. There was an association between low SCC and an increased level of hygiene and frequency of cleaning of the holding yard, passageways and cubicles. Herd management factors associated with bulk tank SCC in Irish grazing herds are generally in agreement with most previous studies from confinement systems of milk production.

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Spatial trends in milk somatic cell count in the Republic of Ireland from 2003 to 2007

Kelly, P.T.[1,2], Collins, D.M.[3], More, S.J.[3], Berry, D.P.[1], Cromie, A.[4], O'Brien, B.[1]

[1] Teagasc Moorepark Dairy Production Research Centre, [2] UCD School of Agriculture, Food Science and Veterinary Medicine,

[3] UCD CVERA, [4] Irish Cattle Breeding Federation Society Ltd.

Ireland produces approximately 5,157 million litres of milk annually which was estimated to be worth €1.4 billion to the Irish economy in 2007. However, milk somatic cell count (SCC) has increased in Ireland in recent years with adverse effects on economic returns both on- and post-farm. Mapping technology may assist in a multi-stranded national approach to tackling this issue, enabling policy-makers to identify geographical regions of high SCC, and facilitating the strategic allocation of finite resources, such as extension services. The objectives of this study were to describe the spatial distribution of herd SCC in milk-recording dairy herds throughout Ireland, to examine if these SCC values cluster, and to determine whether there has been a change in the spatial pattern of these SCC values over time. Herd-year geometric mean SCC values were available for 4,314 milk-recording herds during 2003 to 2007. Each herd was assigned an X and Y coordinate (location on map) based on the centroid of the largest farm fragment. A range of mapping and analysis tools were used including point and kernel density and clustering analysis methods. The south of the country had the greatest density of milk recording herds. Approximately 60% of all herds in the study were from four counties (Cork, Kerry, Limerick and Tipperary). There was an increase in the average bulk tank SCC from 110,264 cells/ml in 2003 to 118,782 cells/ml in 2005, followed by a decrease to 108,454 cells/ml in 2007. Between 2006 and 2007 SCC decreased in the northeast, southeast and west of the country and an increased in the south. There was no spatial clustering of high SCC scores (i.e., SCC on one farm was not related to SCC on other farms), consistent with mastitis being a herd- as opposed to an area-based problem.

The relationship between somatic cell count and parity, stage of lactation, month of calving and age of calving

Kelly, P.T.[1,2], More, S.J.[3], Berry, D.P.[1], O'Brien, B.[1]

[1] Teagasc Moorepark Dairy Production Research Centre, [2] UCD School of Agriculture, Food Science and Veterinary Medicine, [3] UCD CVERA

Grazed pasture constitutes the basal diet of dairy cows in Ireland, with 66% of dairy cows calving between February and April. There is a gap in knowledge of cow factors associated with SCC in a grass based production system. Thus, the objective of the current study was to investigate how SCC differs across parity, stage of lactation, month of calving and age at calving centred within parity within a seasonal calving system where cows are at pasture for the main part of the year. A total of 506,517 test day records from 740 milk recorded herds between 2002 and 2005 were used in this study. Differences in SCC among the factors under investigation were determined using mixed models with cow included as a random effect. The first 305 days post-calving were divided into ten stages of lactation in 30 day increments starting at 5 days in milk (DIM). SCC increased with parity from 97,000 cells/ml in parity 1 to 199,000 cell/ml in parity 6 which could be due to an increased risk of infectious pathogens entering the udder and causing mastitis. SCC decreased between the period 5 to 35 DIM and 36 to 65 DIM, and increased thereafter. The rate of increase in SCS from mid to late lactation was greatest in older parity animals. Cows calving in the months of January and September had lower average SCC. The results from this study can be used in the development of strategies and decision support tools to help benchmark and predict SCC in grass based seasonal production systems.

Association between milk somatic cell count and cow milk yield for different cow parities in a grass based system

Kelly, P.T.[1, 2], O'Sullivan, K.[3], Berry, D.P.[1], More, S.J.[4], O'Brien, B.[1]

[1] Teagasc Moorepark Dairy Production Research Centre, [2] UCD School of Agriculture, Food Science and Veterinary Medicine,

[3] UCC Department of Statistics, [4] UCD CVERA

Subclinical mastitis is one of the most costly diseases of dairy cattle. The cost of this problem may have been over-estimated in the past, as a consequence of the dilution effect of milk yield on somatic cell count (SCC). Some adjustment for the effect of dilution has recently been made, but without considering differences between parities. There are three objectives in the current study: 1) to investigate the association between SCC and milk yield within a seasonal grass based production system, 2) calculate the dilution estimates for each individual parity and 3) use these dilution estimates to investigate the dilution effect of milk yield on SCC. A total of 235,163 test day records from 23,791 cows in 366 Irish milk recorded herds between the years 2003 and 2005 were included in analysis. The association between SCC and milk yield was investigated using two models: 1) the association between both SCC and log_e SCC and milk yield was determined, and 2) four dilution estimates were used to adjust SCC to account for the dilution of SCC by milk yield. A negative association was observed between both SCC and log_e SCC and milk yield. When there was no pre-adjustment of SCC for milk yield, a test day milk loss of 1.43, 2.08, 2.59, 2.56 and 2.62 L was associated with an increase of SCC category from <51 to >400 in parity 1 to 5 animals, respectively. Additionally, two of the dilution estimates had similar trends in milk loss due to SCC to when there was no dilution estimate used. Alternatively, for the pre-adjustment of SCC that multiplied SCC by the mean test day milk yield as a proportion of the test day milk yield, there was an increase in test day milk yield with increasing SCC category. The results from this study can be used to more-accurately calculate the economic implications of SCC.

The relationship between milk somatic cell counts and herd size in Ireland – a retrospective cohort study

Canty, M.J.[1], Binns, S.H.[2]

[1] UCD CVERA, [2] Consultant veterinary epidemiologist

Mastitis is a multi-factorial disease with a long history and results in the impairment of cow welfare due to pain, and has been recognised as a welfare problem in dairy cows. Typically milk somatic cell counts (SCC) are used as an indicator of udder health reflecting the level of inflammation within the udder. SCCs are one of the most important indicators of milk quality, reflecting not only the health status of the udder, but are also the key component of national and international regulation of milk quality. In 2006, the Irish dairy industry produced 5.2 million tonnes of milk, of which approximately 85% was exported as high quality products. SCCs affect the processing potential of milk. EU Council Directive 92/46/EEC requires that raw milk has SCC < 400,000 cells/ml and dairy products sold within the EU must meet these standards. This project will try to determine the relationship between milk somatic cell counts and herd size in Ireland based on data provided by the Irish Cattle Breeding Federation (ICBF).

Development of a HACCP-based approach for the control of mastitis in dairy cows

Beekhuis-Gibbon, L.[1], O'Grady, L.[1], More S.J.[2], Whyte, P.[1, 3], Doherty, M.L.[1]

[1] UCD School of Agriculture, Food Science and Veterinary Medicine, [2] UCD CVERA, [3] UCD Institute of Food and Public Health

Hazard Analysis and Critical Control Points (HACCP) is a systematic, preventative approach that has been developed to increase levels of product safety assurance and which is now widely applied to the dairy processing as well as the retail and catering sectors. A novel approach based on a deconstruction of the infectious process in mastitis was used to develop a generic HACCP-based system to prevent and control mastitis in dairy herds. The approach involved the creation of an Infectious Process Flow Diagram, which was then cross-referenced to two production process flow diagrams of the milking process and cow management cycle, respectively. The generic HACCP plan developed in this study is suitable for customizing and implementation on specific farms. The approach described, offers the potential of a logical, systematic approach to mastitis control that could be used as a template for the control of other infectious diseases of the dairy cow.

Evaluation of the implementation of a HACCP-based approach for the control of mastitis on Irish dairy farms

Beekhuis-Gibbon, L.[1], Devitt, C.[2], O'Grady, L.[1], More, S.J.[3], Whyte, P.[1, 4], Redmond, B.[2], Doherty, M.L.[1]

[1] UCD School of Agriculture, Food Science and Veterinary Medicine, [2] Research consultant, [3] UCD CVERA, [4] UCD Institute of Food and Public Health

As described in an associated paper, a generic HACCP-based programme was developed for the control of mastitis on dairy farms. Subsequently, the programme was customized for, and implemented on, a number of collaborating farms in Ireland, following an initial epidemiological investigation. The aims of the of the present study were to implement customized HACCP-based mastitis control programs on six participating dairy farms, to critically evaluate and obtain feedback from farmers of the actual implementation of the HACCP-based programs, and to obtain sociological insights of farmers into factors associated with the implementation of this program.

Milking parlour. Photograph by S.J. More.

Johne's disease

Risk factors for the introduction and within-herd transmission of *Mycobacterium avium* subspecies *paratuberculosis* (MAP) infection on 59 Irish dairy herds

Cashman, W.[1], Buckley, J.[2], Quigley, T.[3], Fanning, S.[4], More, S.J.[5],

Egan, J.[6], Berry, D.[7], Grant, I.[8], O'Farrell, K.[1]

[1] Private consultant, [2] Cork County Council, [3] Safefood, [4] UCD School of Agriculture, Food Science and Veterinary Medicine, [5] UCD CVERA,

[6] Central Veterinary Research Laboratory, Department of Agriculture, Fisheries and Food, [7] Teagasc Moorepark Dairy Production Research Centre,

[8] Institute of Agri-Food and Land Use, Queen's University, Belfast, Northern Ireland

Since 1994, Irish cattle have been exposed to greater risks of acquiring *Mycobacterium avium* subspecies *paratuberculosis* (MAP) infection as a consequence of the importation of over 70,000 animals from continental Europe. In recent years, there has been an increase in the number of reported clinical cases of paratuberculosis in Ireland. This study examines the prevalence of factors that promote the introduction and within-herd transmission of *Mycobacterium avium* subspecies *paratuberculosis* (MAP) on selected Irish dairy farms in the Cork region, and the association between these factors and the results of MAP screening tests on milk sock filter residue (MFR). A total of 59 dairy farms, selected using non-random methods but apparently free of endemic paratuberculosis, were enrolled into the study. A questionnaire was used to collect data about risk factors for MAP introduction and transmission. The MFR was assessed on six occasions over 24 months for the presence of MAP, using culture and immunomagnetic separation prior to polymerase chain reaction (IMS-PCR). Furthermore, blood samples from all entire male and female animals over one year of age in 20 herds were tested by ELISA. Eighteen (31%) farms had operated as closed herds since 1994, 28 (47%) had purchased from multiple sources and 14 (24%) had either direct or indirect (progeny) contact with imported animals. Milk and colostrum were mixed on 51% of farms, while 88% of farms fed pooled milk. Thirty (51%) herds tested negative to MFR culture and IMS-PCR, 12 (20%) were MFR culture positive, 26 (44%) were IMS-PCR positive and seven (12%) were both culture and IMS-PCR positive. The probability of a positive MFR culture was significantly associated with reduced attendance at calving, and with increased use of individual calf pens and increased (but not significantly) if multiple suckling was practised. There was poor agreement between MFR culture and MFR IMS-PCR results, but moderate agreement between MFR culture and ELISA test results. This study highlights a lack of awareness among Irish dairy farmers about the effect of inadequate bio-security on MAP introduction. Furthermore, within-herd transmission will be facilitated by traditional calf rearing and waste management practices. The findings of viable MAP in the presence of known transmission factors in non-clinically affected herds could be a prelude to long-term problems for the Irish cattle and agri-business generally.

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Demographics of cattle positive for *Mycobacterium avium* subspecies *paratuberculosis* by faecal culture, from submissions to the Cork Regional Veterinary Laboratory

Richardson, E.K.B.[1], Mee, J.F.[1], Sánchez-Miguel, C.[2], Crilly, J.[1], More, S.J.[3]

[1] Teagasc Moorepark Dairy Production Research Centre, [2] Cork Regional Veterinary Laboratory, Department of Agriculture, Fisheries and Food, [3] UCD CVERA

The demography of bovine infections caused by *Mycobacterium avium* subspecies *paratuberculosis* (MAP) in Ireland is poorly defined. The objective of this study was to describe the demographics of cattle positive to MAP on faecal culture, based on submissions to the Cork Regional Veterinary Laboratory (Cork RVL) from 1994 to 2006. The study focused on all available faecal samples from adult cattle with non-responsive chronic diarrhoea that were submitted by private veterinary practitioners to Cork RVL for MAP culture. For each MAP-positive by faecal culture animal, data were collated from Cork RVL and Cattle Movement Monitoring Scheme (CMMS) records. Johne's disease (JD) was confirmed in 110 animals from 86 herds by the Cork RVL between 1994 and 2006, with a rate of positive cases between 15% and 18% over last four years of the study. Two breeds (Holstein/Friesian or Limousin) made up 78% of submissions. Movements were assessed for the 57 study animals with available movement information, 90% died within one year of the test and 26% tested positive in the herd they were born into. The study provides preliminary information about movement trends and demographics of animals with MAP positive submissions. Although the study area is restricted, it includes the most intensive (and economically-important) dairy region in Ireland. The demographics of JD infection from the study area are in agreement with international reports. Further work is required to determine demographic trends, incidence and prevalence of JD throughout Ireland. It is hoped this work may contribute to the development of a surveillance strategy for MAP by regional veterinary laboratories.

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Direct and indirect effects of Johne's disease on farm and animal reproductivity in an Irish dairy farm

Richardson, E.K.B.[1], More, S.J.[2]

[1] Teagasc Moorepark Dairy Production Research Centre, [2] UCD CVERA

Johne's disease (JD) is caused by infection with the organism *Mycobacterium avium* subspecies *paratuberculosis*, leading to chronic diarrhoea and ill thrift in adult cattle. JD is considered to adversely affect farm performance and profitability. This retrospective case study was undertaken on a single commercial dairy herd in the south west of Ireland. Animal production records were interrogated to assess the effect of JD on milk yield (total kg per lactation), somatic cell count (the geometric mean over the lactation), reasons for culling, cull price and changes in herd parity structure over time. JD groups were defined using clinical signs and test results. One control animal was matched to each case animal on parity number and year. Specific lactations (clinical, pre-clinical and test-positive only) from 1994 to 2004 were compared between JD case and control cows. A significantly lower milk yield (1259.3 kg/lactation) was noted from cows with clinical JD in comparison to their matched control group. Clinical animals had an average cull price of €516 less than animals culled without signs of clinical disease. In contrast, little effect was noted for sub-clinical infections. These direct effects of JD infections, in combination with increased culling for infertility and increasing replacement rates, had a negative impact on farm production. Results from this study provide preliminary information regarding the effects of JD status on both herd and animal-level performance in Ireland.

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Prevalence and distribution of paratuberculosis (Johne's disease) in cattle herds in Ireland

Good, M.[1], Clegg, T.A.[2], Sheridan, H.[1], Yearsely, D.[3], O'Brien, T.[3], Egan, J.[3], Mullowney, P.[1]

[1] Department of Agriculture, Fisheries and Food, [2] UCD CVERA, [3] Central Veterinary Research Laboratory, Department of Agriculture, Fisheries and Food

A simple random survey was conducted in Ireland during 2005 to estimate the ELISA-prevalence of paratuberculosis, commonly called Johne's disease (JD), in the cattle population. Serum samples were collected from all 20,322 females/ breeding bulls over 12 months of- age in 639 herds. All samples were tested using a commercially available absorbed ELISA. The overall prevalence of infected herds, based on the presence of at least one ELISA-positive animal, was 21.4% (95% CI 18.4%-24.9%). Herd prevalence levels amongst dairy herds (mean 31.5%; 95% CI: 24.6%, 39.3%) was higher than among beef herds (mean 17.9%; 95% CI: 14.6%-21.8%). However, the animal level prevalence was similar. The true prevalence among all animals tested, was calculated to be 2.86% (95% CI: 2.76, 2.97) and for animals >=2yrs, it was 3.30% (95% CI: 3.17, 3.43). For animals in beef herds, true prevalence was 3.09% (95% CI: 2.93, 3.24), and for those in dairy herds, 2.74% (95% CI: 2.59, 2.90). The majority of herds had only one ELISA-positive infected animal. Only 6.4% (95% CI 4.7%-8.7%) of all herds had more than one ELISA-positive infected animal; 13.3% (CI 8.7%-19.7%) of dairy herds ranging from two to eight ELISA-positive infected animals; and, 3.9% beef herds (CI 2.4%-6.2%) ranging from two to five ELISA-positive infected animals. The true prevalence of herds infected and shedding *Mycobacterium avium* subspecies *paratuberculosis* is estimated to be 9.5% for all herd types; 20.6% for dairy herds; and 7.6% for beef herds. If ELISA positive animals <2-years-of-age are excluded, the true herd prevalence reduces to: 9.3% for all herd types; 19.6% for dairy herds; and 6.3% for beef herds based on a test specificity (Sp) of 99.8% and test sensitivity (Se) (i.e., ability to detect culture-positive, infected animals shedding at any level) of 27.8-28.9%.

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Farming in Ireland. Photograph by S.J. More.

Genetic variation in serological response to *Mycobacterium avium* subspecies *paratuberculosis* and its association with performance in Irish Holstein-Friesian dairy cows

Berry, D.P.[1], Good, M.[2], Mullowney, P.[2], Cromie, A.R.[4], More, S.J.[3]

[1] Teagasc Moorepark Dairy Production Research Centre, [2] Department of Agriculture, Fisheries and Food,

[3] UCD CVERA, [4] Irish Cattle Breeding Federation Society Ltd.

Paratuberculosis, also referred to as Johne's disease, is a contagious and chronic disease in ruminants caused by *Mycobacterium avium* subspecies *paratuberculosis* (MAP). Few estimates of the genetic variation in measures of susceptibility to MAP are available in the literature and even less have attempted to elucidate the genetic associations between measures of susceptibility to MAP and performance in dairy cattle. The objectives of this study were to estimate the genetic variation in serological response to MAP in 4,789 Holstein-Friesian dairy cows from 44 Irish dairy herds, and to quantify its genetic association with performance traits measured in the first three lactations of genetically related animals. Univariate mixed linear and threshold animal models were used to estimate variance components and genetic correlations were estimated using bivariate sire linear mixed models; MAP serological response was treated as a continuous variable and dichotomous variable. The prevalence of MAP in the sample population was 4.8%. This figure cannot be extrapolated to the national dairy herd as the sample population was biased towards herds with increased likelihood of MAP infection. Estimates of heritability for MAP serological response varied from 0.07 to 0.14 depending on the model of analysis and whether serological response was treated as continuous or binary; standard errors varied from 0.024 and 0.062. Genetic correlations between MAP serological response and lactation milk, fat and protein yield were negative although not always more than two standard errors from zero; stronger negative genetic correlations were evident in older parity animals. Serological response to MAP was not genetically correlated with milk fat concentration but was positively genetically correlated with milk protein concentration and negatively correlated with calving interval. Positive genetic correlations existed between MAP serological response and somatic cell count but the correlations were not greater than two standard errors from zero. There was little or no genetic association between serological response to MAP and survival. Results from this study corroborate previous international suggestions that selection for reduced serological response to MAP is possible, although this does not necessarily imply a concurrent selection for either reduced prevalence of clinical disease or increased resistance to MAP infection.

A case-control study of risk factors for paratuberculosis (Johne's disease) in Irish dairy herds

Barrett, D.J.[1], Mee, J.F.[2], Mullowney, P.[3], Good, M.[3], McGrath, G.[4], Clegg, T.A.[4], More, S.J.[4]

[1] Sligo Regional Veterinary Laboratory, Department of Agriculture, Fisheries and Food, [2] Teagasc Moorepark Dairy Production Research Centre,

[3] Department of Agriculture, Fisheries and Food, [4] UCD CVERA

This paper reports a case-control study of herd and management factors associated with the introduction and transmission of paratuberculosis in dairy herds in Ireland. Data were gathered by telephone interview. Case herds were selected on the basis of having one or more positive faecal cultures for *Mycobacterium avium* subsp. *paratuberculosis* (MAP). Control herds were selected from herds seronegative for MAP in a national sero-survey. Control herds were matched to case herds by the year of diagnosis of MAP in the case herds. Factors relating to disease history, herd size, neonatal feeding and management and grassland management were found to be significant (p<0.05) in the univariate analysis. In the multivariate exact logistic analysis, in comparison to control herds the case herds were more likely to have imported cattle from continental Europe, were more likely to have experienced herd depopulation as a result of a regulatory disease outbreak, and were larger in size. With the imminent abolition of milk quotas in the European Union, many Irish dairy herds will be undergoing expansion. Such herdowners need to be aware of paratuberculosis, and take precautions to avoid its introduction and spread. Herdowners unaware of their infection status need to establish it. Where infection is present, management practices must be put in place to control it.

Genetic associations between Johne's disease and susceptibility to *Mycobacterium bovis* and *Mycobacterium avium* subsp *avium* in Irish Holstein Friesian dairy cows

Bermingham, M.L.[1], More, S.J.[2], Good, M.[3], Cromie, A.R.[4], Mullowney, P.[3], Higgins, I.M.[2], Berry, D.P.[1]

[1] Teagasc Moorepark Dairy Production Research Centre, [2] UCD CVERA, [3] Department of Agriculture, Fisheries and Food,

[4] Irish Cattle Breeding Federation Society Ltd.

Johne's disease in cattle is caused by *Mycobacterium avium* subsp. *paratuberculosis* (MAP). A recent study has demonstrated that significant genetic variation exists for susceptibility to MAP infection in Irish Holstein Friesian dairy cows. Nevertheless, data on Johne's disease occurrence is not collected routinely on Irish dairy farms. The objective of this study was to estimate the genetic associations between resistance to MAP infection and measures of susceptibility to *M. bovis* and *M. avium* infection. Serological response to MAP was used as a measure of cow susceptibility to Johne's disease. The single intradermal comparative tuberculin test was used as a measure of susceptibility of cows to *M. bovis* and *M. avium* infection. A total of 4,581 cow serological response to MAP records, 19,663 *M. bovis*-PPD responsiveness records and 15,824 *M. avium*-PPD responsiveness records were available for inclusion in the analysis. Genetic and residual (co)variance components between serological response to MAP and susceptibility to *M. bovis*-PPD and *M. avium*-PPD responsiveness were estimated using bivariate linear animal models. Serological response to MAP was strongly positively genetically correlated (0.84 ± 0.20) with susceptibility to *M. avium*-PPD responsiveness. Susceptibility to *M. avium*-PPD responsiveness was not genetically correlated (0.03 ± 0.32) with serological response to MAP. The results from study suggest that selection for reduced *M. avium*-PPD responsiveness may indirectly increase resistance to MAP infection within the national Holstein Friesian dairy herd.

Infectious Bovine Rhinotracheitis (IBR) / Bovine Viral Diarrhoea (BVD)

Herd and within-herd BoHV-1 prevalence among Irish beef herds submitting bulls for entry to a performance testing station

O'Grady, L.[1], O'Neill, R.[2], Collins, D.M.[3], Clegg, T.A.[3], More, S.J.[3]

[1] Department of Agriculture, Fisheries and Food, [2] Central Veterinary Research Laboratory, Department of Agriculture, Fisheries and Food, [3] UCD CVERA

Infectious bovine rhinotracheitis (IBR), caused by bovine herpes virus 1 (BoHV-1), may result in various clinical consequences, including severe respiratory disease and conjunctivitis, venereal disease and reduced reproductive performance and abortion. This paper presents the serosurveillance findings from an intake of bulls into a performance testing station in Ireland during November 2007. The herd and within-herd BoHV-1 prevalence in 53 Irish beef herds and the risk factors for infection in these herds were determined, among bulls entering a beef performance testing station in Ireland. BoHV-1 status was determined for 41 herds, of which 30 (73.2%) herds were infected and the mean within-herd BoHV-1 prevalence was 28 (±20)%. Multivariate exact logistic modelling revealed increasing numbers of contiguous herds and decreasing percentage of males within the herd as significant risk factors associated with infected herds. These findings highlight the high prevalence of BoHV-1 infection in those Irish beef herds that submitted bulls to this performance testing station, and raise concerns regarding IBR control nationally.

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To determine the prevalence of Bovine Virus Diarrhoea virus (BVDv) infection and Infectious Bovine Rhinotracheitis (IBR) and to establish associations between seroprevalence and cattle herd productivity in the Republic of Ireland

Cowley, B.[1], Clegg, T.A.[2], More, S.J.[2]

[1] Intervet/Schering-Plough Animal Health, [2] UCD CVERA

Infectious diseases such as Bovine Virus Diarrhoea (BVD) infection and Infectious Bovine Rhinotracheitis (IBR) have been identified as key influences in the future success of the Irish dairy and beef industries. Anecdotal evidence suggests that the prevalence of these diseases is high though limited published data exist on national seroprevalence for either disease in either the dairy or beef sector. The objective of this study is to evaluate the seroprevalence of BVD and IBR in dairy and beef cows in the Republic of Ireland. It is also proposed to determine associations between seroprevalence and production, farm location and farm management strategies.

Leptospirosis

Seroprevalence of leptospirosis in Irish beef/suckler herds

Ryan, E.[1], O'Grady, L.[2], More, S.J.[3], Doherty, M.L.[2], Leonard, N.[2]

[1] Private veterinary practitioner, [2] UCD School of Agriculture, Food Science and Veterinary Medicine, [3] UCD CVERA

The objectives of this study were to establish the herd seroprevalence of leptospirosis in unvaccinated suckler/beef herds in the Republic of Ireland, and to establish the individual animal seroprevalence in the Irish suckler/beef breeding stock population. For the purpose of herd selection, the country was divided into six groups with approximately equal numbers of suckler cows, based on the CMMS data from 2000. Herds were then selected at random, from sera that were made available from the Department of Agriculture National Johne's Disease screening study, while aiming to have a roughly equal quantity of herds in each group. In each herd, the number of eligible animals (N; females and entire bulls > 1 year old) to be tested in each herd was calculated as follows:

- Herd Size < 20, N = all eligible animals ;
- Herd Size ≥ 20, N = 20 (animals chosen at random).

Animals were tested using the Linnodee monoclonal antibody capture ELISA. Initial results were as follows:

- The number of unvaccinated herds tested was 299. Based on the sensitivity and specificity of the ELISA, it was determined that a cut-off of 4 positive animals per herd was required to categorise a herd as positive. This resulted in 276 positive herds (276/299) = 92.3%.
- The total number of unvaccinated individual animals tested was 5054. Of this total, there were 2472 animals positive using the Linodee ELISA (2472/5054) = 48.9%.

Evaluation of the risk factors for leptospirosis in Irish suckler/beef herds

Ryan, E.[1], O'Grady, L.[2], More, S.J.[3], Doherty, M.L.[2], Leonard, N.[2]

[1] Private veterinary practitioner, [2] UCD School of Agriculture, Food Science and Veterinary Medicine, [3] UCD CVERA

The objectives of this study were to establish the epidemiological risk factors affecting herd seroprevalence to *Leptospira hardjo* in unvaccinated suckler/beef herds in the Republic of Ireland. Questionnaires were posted to the herdowners of all those herds sampled in the seroprevalence study. These questionnaires aimed to determine whether the herd was vaccinating for leptospirosis, as well as posing a number of questions concerning disease history on-farm; animal details in the herd; the presence of a stock bull; access to watercourses; the presence of sheep on the farm; biosecurity in the herd and details on animal housing. In order to fully determine the vaccination status of each herd, follow-up telephone questionnaires were undertaken for those herds that did not return a completed questionnaire. Questionnaires were returned from 136 unvaccinated herds. Questionnaire results were completed to varying degrees making statistical analysis more difficult. The only risk factor that affected herd seroprevalence to *Leptospira hardjo* with statistical significance was herd size. Larger herds were associated with a significantly increased herd seroprevalence.

A detailed farm investigation

A randomised controlled study to determine the effect of location on growth, haematological parameters, serum biochemistry, nutritional and endocrinological status, immune function and disease status in housed weanling cattle between January and May 2007

Lane, E.A.[1], Canty, M.J.[1], Clegg, T.A.[1], More, S.J.[1]
[1] UCD CVERA

Long-term problems of cattle performance have been observed on a farm (the 'index farm') in Co. Kilkenny, Ireland. The objective of this study was to identify, if possible, any underlying mechanisms of poor animal performance on this farm, thereby providing possible clues for the ongoing problems that were, or still are, being observed. Some 46 cattle were enrolled in the study from the index farm and a further 31 control cattle were sourced from a farm in Co. Meath. These cattle were divided into five separate treatment groups, each being supplied with defined feed and water combinations. The index farm cattle had lower initial weights than the control cattle but were matched in all other respects. Three of these groups were based on the index farm and two were resident on a control farm located approximately five km away. Throughout the study, the animals were weighed, bled and assessed at regular intervals. An extensive battery of laboratory testing was undertaken to assess factors that could potentially be linked to poor growth, including nutrition (energy status, an assessment of elemental status), disease and immunity (serology, serum biochemistry, general immune status) and endocrine parameters associated with growth. Analysis of feed and water was also conducted. The animals were vaccinated, and treated for internal parasites, at the start of the study. All cattle performed well throughout the study period, thereby limiting the ability of this study to identify any causes of the ongoing intermittent poor performance problems. During January to May 2007, the index farm and control cattle achieved ADGs of 0.66 and 1.00 kg/day, respectively. Among the many parameters assessed, few significant differences were identified within and between groups. Mineral deficiencies/imbalances had been identified in the past, and were comprehensively assessed and described in this study. At the start and throughout the study, the cattle from the index farm were selenium (Se) deficient. Selenium also decreased among the control cattle, reaching the threshold for Se deficient status towards the end of the study. Results from this study indicated that Se deficiency may be a contributor to the ongoing performance problems on the farm. IGF-1, a principal mediator of growth hormone in animals, was markedly lower in the index sourced cattle at the start of the trial, though the concentrations increased in both groups throughout the period. Cadmium (Cd) was measured in cattle towards the end of the study, the significance of which is uncertain.

An evaluation of underlying performance mechanisms in an experimental group of animals kept on the index farm from January 2007 to April 2008

Lane, E.A.[1], Canty, M.J.[1], Clegg, T.A.[1], More, S.J.[1]
[1] UCD CVERA

Long-term problems of cattle performance have been observed on a farm (the 'index farm') in Co. Kilkenny, Ireland. The objective of this study was to determine whether periods of poor weight gain or weight loss, if observed, were associated with changes in haematological parameters, essential element status, or exposure to heavy metals. Cattle (n = 10) sourced off farm were moved to the index farm in January 2007 (Day 0; 16/01/2007) and kept there until April 2008 (day 469; 29/04/2008). Blood-samples were collected by jugular venepuncture and cattle were weighed on Days 0, 112, 154 (blood-sample only), 203, 231, 259, 287, 315, 357, 386, 413, 442 and 469 relative to the start of the study. Weight gains were intermittent during the period of observation: very good ADGs were observed during the initial housing period (January to May 2007; 0.96 kg/day), very poor during summer grazing (May to October 2007, 0.13 kg/day) and good during the subsequent housing period (October 2007 to April 2008, between 0.55 and 1.2 kg/day). During the

summer grazing period, the cattle experienced a coincident reduction in weight gain, which is suggestive of a single insult over a defined period of time. At this time, there was no overt evidence of disease in these cattle. Adequate ADGs during summer 2007 have been reported elsewhere in Ireland. Selenium concentrations declined during the study period and were considered to be deficient from October 2007 to January 2008. However, the concentrations improved on winter feeding and returned to normal by February 2008. Copper (Cu) concentrations declined after cattle were introduced onto the index farm, and were considered deficient from May 2007 until the end of the study. In this study, essential element deficiencies were again identified as potential contributors to the ongoing performance problems on the index farm. A single Cd peak was measured in cattle during this study, the significance of which is uncertain but subject to further study.

An assessment of responsiveness of calves from the index farm, with known essential element deficiencies to selenium supplementation, May 2007 to April 2008

Canty, M.J.[1], Lane, E.A.[1], Clegg, T.A.[1], Sharpe, A.[2], More, S.J.[1]

[1] UCD CVERA, [2] Central Veterinary Research Laboratory, Department of Agriculture, Fisheries and Food

Long-term problems of cattle performance have been observed on a farm (the 'index farm') in Co. Kilkenny, Ireland. Earlier studies have indicated that selenium (Se) deficiency may be contributing to this problem. The aims of this study were to evaluate the efficacy of different forms of selenium supplementation to maintain the Se status and perform-ance of animals, and to compare the performance of Se supplemented animals with un-supplemented animals moved to an unaffected farm. In a randomised controlled study Friesian cross calves (n = 42) were assigned to one of four groups; *Tx1OnFC* 2ml sc injection sterile water, remained on the index farm, n = 11. *Tx2BVP* sc injection BVP Barium Selenate LA® (1 mgSe/kg LBW), remained on the index farm, n = 10. *Tx3Vitasel* im injection of Vitasel® (68mgVit E/ml & 1.5mgSe/ml) at 2ml/45kg LBW, remained on the index farm, n = 11. *Tx4OffFC* 2ml sc injection of sterile water, animals remained on the index farm for 41 days, moved to unaffected farm for 125 days, subsequently returned to the index farm, n=9. All animals had normal Se and GPx concentrations at the start of the study on Day 0, however, Se and glutathione peroxidase GPx concentrations declined in all groups until Day 307 and Day 280, respectively. Se concen-trations were below normal (120 µg/kg) in *Tx1OnFC* and *Tx3Vitasel* at 13 of the 21 sampling points, and in 7 of the 21 sampling point in *Tx4OffFC*, following their return to the index farm. *Tx2BVP* had higher Se and GPx concentrations compared with *Tx3Vitasel*, however, neither form of Se supplementation was sufficient to overcome the poor average daily gains (ADG) on the index farm. Following acclimatisation, *Tx4OffFC* animals, moved to the unaffected farm had improved ADG and normal Se concentrations, but both declined upon return to the index farm. The Se deficit status of the index farm was confirmed by the movement of animals off, and subsequently back to the index farm, confirming the localised nature of the deficiency. Selenium supplementation had a positive impact on blood Se status, but was not sufficient to overcome the shortfall in animal performance, suggesting that Se deficiency may be just a component of a more complex interaction. In support of this view, marginal to deficient concentrations of Cu and iodine (I) were also observed in all groups. The presence of radiodense growth retardation lines is consistent with poor calf growth, but does not provide any definitive insight into the cause of the problem. Two patterns of Cd excess were observed during this study: a large Cd peak and background Cd exposure. The significance of these findings is uncertain but subject to further study. Understanding the potential complex interaction between element excesses and deficiencies on the index farm could prove important.

A survey of essential elements and heavy metals in a small area encompassing the index farm, April and September 2007

Canty, M.J.[1], McCormack, S.[2], Lane, E.A.[1], Collins, D.M.[1], More, S.J.[1]

[1] UCD CVERA, [2] Teagasc Johnstown Castle Research Centre

Many ruminants are solely or mostly dependant for all their nutrients, including essential elements, on the quality of the forage available to them, either in its natural state or conserved as hay or silage. Shortfalls in animal performance, principally stunted growth and ill-thrift in growing cattle but also poor body condition and reduced milk yield in adult cattle, has also been observed on a farm (index farm) in Castlecomer, Co. Kilkenny, Ireland. A soil and herbage survey was carried out over two time periods, April 2007 and September 2007, in a 2.5 sq km grid incorporating sampling points on the index farm (at its centre) and on 27 other farms in the area. In this study, we sought to determine the nutrient and heavy metal status of soil and herbage in the sampling area, and to relate the concentrations reported with their potential to impact on animal performance. Low soil pH and high soil liming requirements were identified within the sampling area and on the index farm, noting that soil pH greatly influences nutrient uptake by plants from soil, and subsequently nutrient availability to grazing animals. Calcium (Ca), copper (Cu), selenium (Se) and zinc (Zn) concentrations were low in soils and herbage in the sampling area and on the index farm. These conditions are not dissimilar to those found on other farms in Ireland. Fluoride (F) was detected in 61 of the 97 herbage samples in April 2007, but only four exceeded 40 mg/kg (the maximum tolerable level) for cattle. No measurement of Cd was undertaken in herbage samples. In soil samples, Cd was below the level of detection for the test used (<0.1 mg/kg) in over 90% of samples tested, and those where Cd was reported, the maximum Cd concentration was 0.72 mg/kg, within the typical range for Irish soils (0.1 to 1 mg/kg). Mineral imbalances (Ca, Cu, Se and Zn) in pastures caused by low soil mineral status, exacerbated by low soil pH, could hinder optimal animal performance in the region.

Kidney cadmium concentrations in cattle from the index farm, 2003-2005 and 2009

Canty, M.J.[1], Lane, E.A.[1], More, S.J.[1]

[1] UCD CVERA

Of all animal tissues, livers and kidney constitute a special dilemma in that they have a tendency to bioaccumulate toxic metals such as arsenic (As), cadmium (Cd), lead (Pb) and mercury (Hg). Maximum concentrations (ML) for Hg, Pb, Cd and tin (Sn), but not As, in foodstuffs are set by European Commission Regulations. The aims of this study were 1) to determine the concentrations of As, Cd, Pb and Hg in the livers and kidneys of nine cows from the index farm and 2) to combine the kidney Cd results from these 5 cows with those reported in an earlier Department of Agriculture, Fisheries and Food (DAFF) report (n = 14 animals), and describe the trend in kidney Cd concentrations on the index farm over time. Arsenic, Pb and Hg concentrations in livers and kidneys were within normal range for animal health purposes for the nine cows. Kidney Cd concentrations in six of the nine cows exceeded the normal range for animal health purposes (0.05-1.5 mg/kg) and seven of the nine exceeded the ML permitted for human consumption (1 mg/kg). When kidney Cd results from the nine cows (2009) were combined with results (n = 14) from a previous investigation (2003-2005), 8 of the 23 (37.8 %) kidneys had Cd concentrations above 1.5 mg/kg, the upper limit of the normal range for animal health. Kidney Cd concentrations appear to increase in animals with increasing time spent on the index farm. Based on current knowledge, exposure at this level is not associated with adverse effects in terms of animal health or performance.

An epidemiological evaluation of all relevant data gathered to date and an assessment of hypotheses that could plausibly be associated with poor animal performance on the index farm

More, S.J.[1], Lane, E.A.[1], Canty, M.J.[1], McGrath, G.[1], Sheridan, M.[2]

[1] UCD CVERA, [2] Department of Agriculture, Fisheries and Food

Long-term problems of cattle performance were observed on a farm (the 'index farm') in Co. Kilkenny, Ireland. In recent years, a number of investigations have been conducted. The objectives of this paper are to critically evaluate all relevant data, and to assess hypotheses that could plausibly be associated with these problems of animal performance. A number of general hypotheses were developed, each potentially associated with case development, including hypotheses specific to the index farm (health, management, genetics, nutrition) and broader hypotheses (element imbalances). Each hypothesis was then assessed by considering all available sources of data, taking account of data validity and precision, and using the Bradford-Hill criteria for evidence of causation during decision-making. A number of essential element deficiencies have been identified in soil, herbage and cattle on the index farm, including calcium, copper, selenium and zinc. Observed soil conditions (low pH, high liming requirements) are known to adversely affect the availability of essential elements. The deficiency in cattle was not responsive to Se supplementation, consistent with an aetiology more complex than Se alone. A number of animal health concerns were identified on the index farm prior to 2007, with adverse effects on animal performance. Since 2007, however, the potential for such effects has been substantially reduced, following the introduction of robust preventive measures and as a consequence of intensive veterinary supervision by the project team and the farmer's own private veterinary practitioners. There is evidence of elevated background concentrations of Cd among cattle on the index farm. However, these concentrations are not of toxicological significance based on currently knowledge, and would not be expected to adversely affect animal health. Fluorine is present intermittently, but not at concentrations considered detrimental to animal health. A number of factors have been described that undoubtedly have some influence on the performance of cattle on the index farm. However, no comprehensive understanding has yet been established to fully explain the epidemiological presentation of cases, in particular the localisation of cases to the index farm and the temporal distribution of periods when negative performance was recorded.

HERD HEALTH
A detailed farm investigation

Other animal health issues

Brucellosis

An evaluation of Irish cattle herds with inconclusive serological evidence of bovine brucellosis

Hayes, M.[1,2], Ashe, S.[1,2], Collins, D.M.[2], Power, S.[3], Kenny, K.[4], Sheahan, M.[1], O'Hagan, G.[1], More, S.J.[2]

[1] Department of Agriculture, Fisheries and Food, [2] UCD CVERA, [3] Blood Testing Laboratory, Department of Agriculture, Fisheries and Food,

[4] Central Veterinary Research Laboratory, Department of Agriculture, Fisheries and Food

Since 1998, there has been a steady decline in herd restrictions and de-populations in Ireland due to bovine brucellosis. There is concern that the interpretation of laboratory results may become increasingly problematic, as brucellosis prevalence falls in Ireland. Therefore, the purpose of the current study was to evaluate the infection status of Irish herds and animals with inconclusive serological evidence of bovine brucellosis. During 12 months from September 1, 2004, laboratory and observational epidemiological data were collected from all Irish herds where animal testing identified at least one animal with a complement fixation test (CFT) reading greater than zero and/or a positive result to the indirect enzyme-linked immunosorbent assay (iELISA). Due to the observational nature of the study, we have robust estimates of the relative, but not the absolute, performance of the CFT, iELISA and brucellin skin test (BST). Herds were divided into three categories (Group A, B or C) on the basis of test results at initial assessment. A total of 639 herds were enrolled into the study, and observed for at least two years following enrolment. A rising CFT titre, with a CFT reading of 111 International CFT Units (IU) or greater at the subsequent blood test, was generally associated with herds where other evidence of infection was also available. Knowledge of the CFT reading at the initial and a subsequent blood test proved useful in distinguishing false-positive and true-positive brucellosis results. There was poor correlation between the CFT and iELISA results, and between the CFT and BST results. As a result of this study, national policy has been modified to include re-sampling of all animals with CFT readings of 20 IU or greater. This project has also led to a reduction in the number of herds restricted, as well as restriction duration. It has also contributed to a reduction in the number of herds listed for contiguous tests, and therefore the potential for contiguity testing of false positive results.

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Outbreak of bovine brucellosis in County Clare, Ireland, in 2005

Hayes, M.[1,4], Kilroy, A.[1], Ashe, S.[1,4], Power, S.[2], Kenny, K.[3], Collins, D.M.[4], More, S.J.[4]

[1] Department of Agriculture, Fisheries and Food, [2] Blood Testing Laboratory, Department of Agriculture, Fisheries and Food,

[3] Central Veterinary Research Laboratory, Department of Agriculture, Fisheries and Food, [4] UCD CVERA

This paper describes an investigation of an outbreak of bovine brucellosis in County Clare, Ireland, during 2005. It is likely that infection on the index farm was linked to a previous outbreak of brucellosis in County Clare. During March to May 2005, transmission of brucellosis within the herd was rapid; this was facilitated by a range of factors, including close contact between cattle kept in winter housing, and the mixing of animals, both during grazing and at housing, throughout the year. Containment of the disease, including only limited spread to one contiguous herd, was facilitated by the recent construction of a shed for winter housing.

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Equine infectious anaemia

Management of the national programme to eradicate equine infectious anaemia from Ireland during 2006: a review

Brangan, P.[1], Bailey, D.C.[1], Larkin, J.F.[1], Myers, T.[1], More, S.J.[2]

[1] Department of Agriculture, Fisheries and Food, [2] UCD CVERA

Ireland experienced an outbreak of equine infectious anaemia (EIA) in 2006. The infection was first detected on 15th June 2006, in a mare following euthanasia at a veterinary hospital. During the following 6 months, a total of 38 cases were detected, in 2 distinct epidemiological clusters (centred on counties Meath and Kildare). The outbreak affected horses from 18 separate home premises in 8 Irish counties (Kildare, Meath, Dublin, Wicklow, Wexford, Limerick, Louth and Monaghan) and one county (Derry) in Northern Ireland. There is growing concern about outbreaks in equine populations (Herholz *et al.* 2008). A number of agents, including equine influenza (EI) and African Horse Sickness (AHS) have the potential for rapid spread within equine populations. Information is available about the management of animal health emergencies in production animals. As yet, there are limited published examples highlighting issues relating to the national management of equine disease emergencies. This paper presents the national response (control and eradication strategies, programme management, linkages with industry and the international community, resource issues) to the 2006 EIA outbreak in Ireland and lessons learned.

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An outbreak of equine infectious anaemia in Ireland during 2006: investigation methodology, initial source of infection, diagnosis and clinical presentation, modes of transmission and spread in the Meath cluster

More, S.J.[1], Aznar, I.[1], Bailey, D.C.[2], Larkin, J.F.[2], Leadon, D.P.[3],
Lenihan, P.[4], Flaherty, B.[2], Fogarty, U.[3], Brangan, P.[2]

[1] UCD CVERA, [2] Department of Agriculture, Fisheries and Food, [3] Irish Equine Centre,

[4] Central Veterinary Research Laboratory, Department of Agriculture, Fisheries and Food

Equine infectious anaemia (EIA) was confirmed in Ireland on 15th June 2006. Over the following 6 months, until 10th December 2006, a total of 38 EIA cases were identified. No further cases have been identified, despite ongoing surveillance, suggesting that the infection has been successfully eradicated. This was the first outbreak of this disease in Ireland with evidence of transmission of infection. Infectious disease outbreaks are of considerable concern to the international equine industry, and a number of agents (e.g. equine influenza, African horse sickness) have the potential for rapid spread within equine populations. Despite its importance, however, there have as yet been few reports in international peer-reviewed journals of outbreak investigations in equine populations, either in Europe or elsewhere. This paper describes the investigation of the equine infectious anaemia outbreak in Ireland during 2006, with emphasis on the investigation methodology, initial source of infection, aspects of diagnosis and clinical presentation during the outbreak, and modes of transmission and spread in the Meath cluster.

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An outbreak of equine infectious anaemia in Ireland during 2006: the modes of transmission and spread in the Kildare cluster

More, S.J.[1], Aznar, I.[1], Myers, T.[2], Leadon, D.P.[3], Clegg, T.A.[1]

[1] UCD CVERA, [2] Department of Agriculture, Fisheries and Food, [3] Irish Equine Centre

Equine infectious anaemia (EIA) was confirmed in Ireland on 15th June 2006. During the following 6 months, until 10th December 2006, a total of 38 EIA cases were detected, including a single case in Northern Ireland. These cases were linked within 2 clusters, centred in counties Meath and Kildare. The Meath cluster was primarily related to 'Veterinary Practice X and its associated clinic' and the Kildare cluster to 'Veterinary Hospital Y' (subsequently termed 'the hospital'). Aspects of this outbreak have been presented elsewhere, including an overview of the outbreak (Brangan *et al.* 2008), information about the initial source of infection, aspects of diagnosis and clinical presentation during the outbreak, and of the modes of transmission and spread within the Meath cluster (More *et al.* 2008). This paper presents the findings of an epidemiological investigation of the Kildare cluster, with emphasis on the mode(s) of transmission and spread of infection.

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Transmissible spongiform encephalopathy (TSE)

Confirmed BSE cases in Ireland during 2008
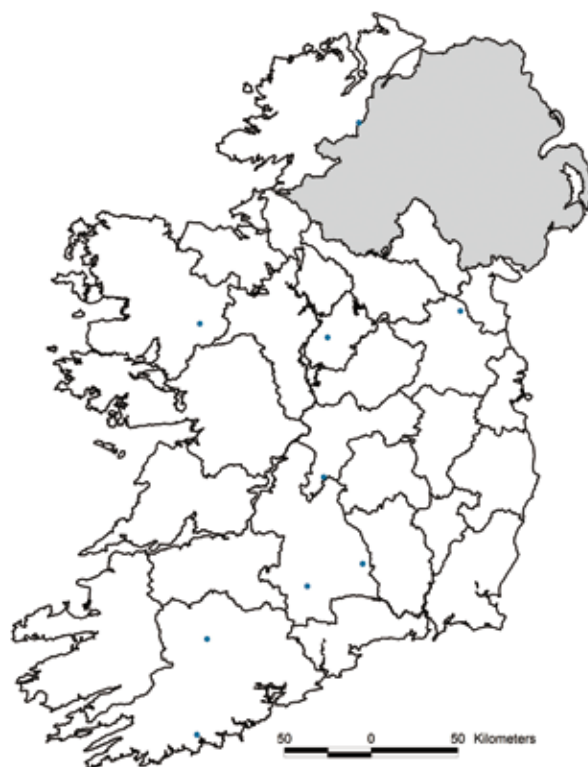
Confirmed BSE cases in Ireland during 2009

Avian influenza

A review of Ireland's waterbirds, with emphasis on wintering migrants and reference to H5N1 avian influenza

Crowe, O.[1], Wilson, J.[2], Aznar, I.[3], More, S.J.[3]

[1] Birdwatch Ireland, [2] National Parks and Wildlife Service, [3] UCD CVERA

Ireland is characterised by its diversity and large abundance of wetlands, making it attractive to a wide variety of water-birds throughout the year. This paper presents an overview of Ireland's waterbirds, including ecological factors relevant to the potential introduction, maintenance, transmission and spread of infectious agents, including the H5N1 avian influenza virus, in Ireland. Particular emphasis is placed on five groups of wintering migrants (dabbling and sieving wild-fowl, grazing wildfowl, diving wildfowl, waders and gulls), noting that the H5N1 avian influenza virus has mainly been isolated from this subset of waterbirds. Ireland's wetlands are visited during the spring and summer months by hundreds of thousands of waterbirds which come to breed, predominantly from southern latitudes, and during the autumn and winter by waterbirds which come from a variety of origins (predominantly northern latitudes), and which are widely distributed and often congregate in mixed-species flocks. The distribution, feeding habits and social interactions of the five groups of wintering migrants are considered in detail. Throughout Ireland, there is interaction between different waterbird populations (breeding migrants, the wintering migrants and resident waterbird populations). There is also a regular and complex pattern of movement between feeding and roosting areas, and between wetlands and farmland. These interactions are likely to facilitate the rapid transmission and spread of the H5N1 avian influenza virus, if it were present in Ireland.

A qualitative assessment of the risk of spread to and within the commercial poultry industry, following the introduction of H5N1 avian influenza into Ireland, and of potential risk mitigation measures

Aznar, I.[1], Crowe, O.[2], Wilson, J.[3], Duignan, P.J.[4], Gaynor, S.[4], Neilan, R.[4], McLoon, D.[5], McArdle, P.J.[4], More, S.J.[1]

[1] UCD CVERA, [2] Birdwatch Ireland, [3] National Parks and Wildlife Service, [4] Department of Agriculture, Fisheries and Food, [5] Monaghan Veterinary Consultants

As required under EU legislation (European Commission, 2005a), Ireland has developed an Avian Influenza (AI) opera-tional manual and contingency plan. Relevant to ongoing preparations for an AI incursion, this paper describes work to qualitatively assess the risk of spread to and within the commercial poultry industry, following the introduction of H5N1 avian influenza into Ireland, and of potential risk mitigation measures. Four transmission routes were considered, including spread among wild waterbirds, spread from wild waterbirds to non-commercial avian operations, spread from wild waterbirds to commercial poultry (directly or via non-commercial avian operations) and spread within the commer-cial poultry industry. Data for the assessment was drawn from the scientific literature, from national expert opinion, from national databases and unpublished national reports. There is considerable potential in Ireland for spread of H5N1 avian influenza among wild waterbirds, and from wild waterbirds to non-commercial avian operations. In contrast, the opportunity for spread to and within the commercial poultry sector is variable, depending on a broad range of factors including production system, water source and management. A number of important risk mitigation measures were identified, focusing on improved information and awareness, risk based surveillance and maintenance of up-to-date data-bases. Each of these issues has now been considered in detail, both by DAFF and industry.

Animal movement and population structure

Survival and dispersal of a defined cohort of Irish cattle

Ashe, S.[1,2], More, S.J.[2], O'Keeffe, J.[1,2], White, P.[1,2], McGrath, G.[2], Aznar, I.[2]

[1] Department of Agriculture, Fisheries and Food, [2] UCD CVERA

An understanding of livestock movement is critical to effective disease prevention, control and prediction. However, livestock movement in Ireland has not yet been quantified. This study has sought to define the survival and dispersal of a defined cohort of cattle born in Co. Kerry during 2000. The cohort was observed for a maximum of four years, from January 1, 2000 to December 31, 2004. Beef and dairy animals moved an average 1.31 and 0.83 times, respectively. At study end, 18.8% of the beef animals remained alive on Irish farms, including 6.7% at the farm-of-birth, compared with 48.6% and 27.7% for dairy animals respectively. Beef animals were dispersed to all Irish counties, but mainly to Cork, Limerick, Tipperary and Galway. Dairy animals mainly moved to Cork, Limerick, and Tipperary, with less animals going to Galway, Meath and Kilkenny. The four-year survival probability was 0.07 (male beef animals), 0.25 (male dairy), 0.38 (female beef), and 0.72 (female dairy). Although there was considerable dispersal, the number of moves per animal was less than expected.

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Trends in cow numbers and culling rate in the Irish cattle population, 2003 to 2006

Maher, P.[1], Good, M.[1], More, S.J.[2]

[1] Department of Agriculture, Fisheries and Food, [2] UCD CVERA

Cows are the main economic production units of Ireland's cattle industry. Therefore, demographic information, including overall numbers and survival rates, are relevant to the Irish agricultural industry. However, few data are available on the demographics of cows within a national population, either in Ireland or elsewhere, despite the recent development of comprehensive national cattle databases in many EU Member States. This study has sought: to determine the rate of cow culling from the national herd; to determine the rate of culling by type (dairy, beef), age, method of exit, date of exit and interval between last calving and exit; to calculate the national cow on-farm mortality rate; and to compare the Irish rates with published data from other countries. This work was conducted using data recorded in the national Cattle Movement Monitoring System (CMMS). Culling refers to the exit of cows from the national herd, as a result of death but regardless of reason, and cow-culling rate was calculated as the number of cow exits (as defined above) each year divided by the number of calf births in the same year. Culling rate was determined by type (dairy or beef), date of birth, method of exit (slaughter or on-farm death), month of exit and interval between last calving and exit. The average cow-culling rate during 2003 to 2006 was 19.6% (21.3% for dairy, 18% for beef). While comparisons must be treated with caution, it concluded that the overall rates of culling in Ireland fell within published internationally accepted norms. The on-farm mortality rate of 3.2-4.1% was similar to that reported in comparable studies.

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Modelling the demographics of the Irish cattle population

O'Connor, J.[1, 2], More, S.J.[2], Griffin, J.M.[1], O'Leary, E.[3]

[1] Department of Agriculture, Fisheries and Food, [2] UCD CVERA, [3] UCD School of Mathematical Sciences

In recent years, national authorities have committed very substantial resources to the creation and maintenance of databases capable of recording important animal event data, such as births, deaths and movements. This has primarily been driven by the need to ensure the quality and safety of animal products. However, it can also be used to assist policy-makers in decision making. Despite the abundance of animal event data, as yet there is little published information about the use of these data to better understand the demography of cattle populations. This study reports the development of, and outputs from, a demographic model using data routinely collected from the Irish cattle population. The demographic model was based on a series of life tables detailing age-specific probabilities of survival up to a maximum of 17 years. These outputs were used to determine characteristics of the Irish cattle population, including estimated mortality rates, life expectancies and age profiles, and estimated cattle numbers by age and date. Separate life tables were developed for each of the 204 monthly birth cohorts born between January 1989 and December 2005. Within the Irish cattle population, the peak estimated mortality rate occurs at 29–33 months. The estimated life expectancy at birth of cattle in Ireland was 42 months. When the survival rates for all the cohorts within a population are calculated, then it is possible to use these rates as a model for determining future population size and answering cohort specific queries.

OTHER ANIMAL HEALTH ISSUES
Animal movement and population structure

Companion animal epidemiology

Demography of the pet dog and cat population on the island of Ireland and human factors influencing pet ownership

Downes, M.[1], Canty, M.J.[1], More, S.J.[1]

[1] UCD CVERA

Published data on aspects of domestic pet demographics are available in many countries. Several of these studies have linked household demographics, such as the presence of children in the household, to pet ownership. There is very little published information about the demography of domestic pets on the island of Ireland (the Republic of Ireland, Northern Ireland). This study was conducted to describe the demography of the pet dog and cat populations on the island of Ireland and to identify human factors influencing pet ownership. A questionnaire was designed and administered to households to collect data about the demographics of households and their dogs and cats. The questions related to location, building structure, social class, nationality and family structure of the household, and the sex, age and source of each pet dog and/or cat. The survey was administered by a commercial company, using computer-assisted telephone interview techniques to 1250 households selected using random digit dialling and quota controls. In this study, a pet dog was defined as a dog that was been fed by a household and considered a pet by the participant of the study. A pet cat was defined as a cat that was both fed by the household and allowed into the house. The results show that 35.6% of households in Ireland have one or more pet dogs and 10.4% of households have one or more pet cats. In total, 47.3% of pet dogs and 76.1% of pet cats were neutered. Females of both species are more likely to be neutered than males. Factors associated with dog ownership included location, house type, household social class, household composition, the presence of school children in the house, and the presence of a cat in the house. Factors associated with pet cat ownership included the type of house structure, the presence of a dog in the house and the gender and age of the participant. Cats tend to stray into households. This study was the first to provide detailed information about the demographics of the pet dog and cat populations on the island of Ireland, and has identified areas for further research, in particular the effect of stray dogs and cats on the owned pet dog and cat population, and of future demographic trends in these populations.

Factors associated with pet ownership among patients with asthma

Downes, M.[1], Roy, A.[2], Wisnivesky, J.P.[3]

[1] UCD CVERA, [2] Department of Pediatrics, Mount Sinai School of Medicine, New York, USA, [3] Division of General Internal Medicine and Pulmonary, Critical Care and Sleep Medicine, Department of Medicine, Mount Sinai School of Medicine, New York, USA

Exposure to indoor allergens is an established risk factor for poor asthma control. Current guidelines recommend removing pets from the home of patients with asthma. This study was conducted to identify factors associated with pet ownership in asthma sufferers. Using data from The National Asthma Survey, we carried out univariate and multiple regression analyses to identify independent predictors of pet ownership in asthma sufferers after controlling for potential confounders. Overall, people with asthma were more likely to own a pet (49.9% vs. 44.8% p < 0.001). Additionally, 68.7% of patients with asthma who own a pet allowed them into their bedroom. Multivariate analysis showed that female sex, adults, white race and high income were independent predictors of pet ownership among

asthmatics. Higher income and having only an adult with asthma in the home were also associated with increased likelihood of allowing a pet into the bedroom. Pet ownership is common among asthmatics and the majority of patients allow their pets into their bedroom. Addressing this problem may help improve asthma morbidity.

Pet owner attitudes towards ownership, neutering and nutrition of their pets

Downes, M.[1], McKenzie, K.[2], More, S.J.[1]

[1] UCD CVERA, [2] UCD Geary Institute

The aim of this project is to generate new insights and hypotheses into the reasons for pet ownership, neutering and owners attitudes towards obesity and nutrition. Focus groups were deemed the appropriate method for data collection. Because little previous research has examined the attitudes and beliefs underlying human-pet interactions, qualitative methods employing a bottom-up approach will be used to generate data driven by participants.

Spatial analysis of the pet dog and cat population of Ireland

Downes, M.[1], More, S.J.[1]

[1] UCD CVERA

Using geographical information systems (GIS) and probability data obtained from the study entitled 'Demography of the pet dog and cat population on the island of Ireland and human factors influencing pet ownership' we are matching geographical positioning data with Census 2006 data and socio-demographic influencing pet ownership to produce a spatial distribution map of the pet population of Ireland.

Photograph by M.J. Canty.

Aquatic animals

An introduction to import risk analysis for aquatic animals

Baldock, F.C.[1], More, S.J.[2], Peeler, E.J.[3]

[1] AusVet Animal Health Services, Queensland, Australia, [2] UCD CVERA,

[3] Centre for Environment, Fisheries and Aquaculture Science, Dorset, United Kingdom

With increasing international trade, there are increasing risks to countries that unwanted aquatic animal pathogens will enter and spread. Import risk analysis (IRA) provides an objective, transparent and defensible method of assessing disease risks associated with imports. The International Aquatic Animal Health Code provides internationally recognised guidelines for import risk analysis. This paper describes and illustrates the main elements of an IRA for aquatic animals and their products, including hazard identification, risk assessment, risk management and risk communication. Sources of additional information are listed, both on concepts and methodology, and also the application of import risk analysis in aquatic animal health management.

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Animal welfare

Equine welfare

The structure and regulation of the Irish equine industries: links to considerations of equine welfare

Collins, J.[1], Hanlon, A.[2], More, S.J.[1], Duggan, V.[2]

[1] UCD CVERA, [2] UCD School of Agriculture, Food Science and Veterinary Medicine

The equine industries in Ireland are vibrant and growing. They are broadly classified into two sectors: Thoroughbred racing, and sports and leisure. This paper describes these sectors in terms of governance, education and training in equine welfare, and available data concerning horse numbers, identification, traceability and disposal. Animal welfare, and specifically equine welfare, has received increasing attention internationally. There is general acceptance of concepts such as animal needs and persons' responsibilities toward animals in their care, as expressed in the 'Five Freedoms'. As yet, little has been published on standards of equine welfare pertaining to Ireland, or on measures to address welfare issues here. This paper highlights the central role of horse identification and legal registration of ownership to safeguard the health and welfare of horses.

Policy Delphi with vignette methodology as a tool to evaluate the perception of equine welfare

Collins, J.[1], Hanlon, A.[2], More, S.J.[1], Wall, P.G.[3], Duggan, V.[2]

[1] UCD CVERA, [2] UCD School of Agriculture, Food Science and Veterinary Medicine, [3] UCD Geary Institute

A three-round Policy Delphi using vignette methodology was employed as a new approach to study stakeholder perceptions and experiences of equine welfare. Forty-four representatives from stakeholder groups in the Irish equine industry participated. In Round 1, vignettes (narratives illustrating potential infringements of equine welfare) were presented to assess perceptions of 'Acceptability' and experiences of 'Frequency of occurrence'. In Round 2, lists of situations where equine welfare might be compromised, possible drivers of behaviour and potential solutions were presented for grading. In Round 3, two composite issues were formed from an analysis of responses to the previous round, namely (1) the disposal of horses trade, and (2) behaviour at unregulated gatherings; these were illustrated using vignettes to establish stakeholder attitudes to the desirability, feasibility and means of improving standards of welfare for horses. All respondents completed all rounds demonstrating their engagement with the method.

Evaluation of current equine welfare issues in Ireland: causes, desirability, feasibility and means of raising standards

Collins, J.[1], Hanlon, A.[2], More, S.J.[1], Wall, P.G.[3], Kennedy, J.[3], Duggan, V.[2]

[1] UCD CVERA, [2] UCD School of Agriculture, Food Science and Veterinary Medicine, [3] UCD Geary Institute

Significant potential threats to the health and welfare of horses exist in Ireland when supply exceeds demand and the identification system for horses is not yet robust. This study was conducted to secure engagement with stakeholder groups and to determine their perception of equine welfare in Ireland and to encourage the development of inclusive, rather than imposed, policy solutions. A 3 round, web-based Policy Delphi incorporating novel vignette methodology was conducted from November 2007–March 2008 to canvass opinion (in both quantitative and qualitative forms) on the perceived most significant equine welfare issues. Vignettes (narratives depicting potential compromise to equine welfare) were employed. Quantitative data were collected in the form of scoring on a 9 point Likert scale with labelled end-points, qualitative information as text subsequently analysed for themes. All 44 respondents completed all rounds. Major equine welfare issues were identified as welfare of horses during the disposal process and at unregulated gatherings. Assessed quantitatively on a 9 point Likert scale (0 = minimal; 8 = maximal), respondents scored the desirability and feasibility of improving standards, median 8 and 6, respectively, for both issues identified. Basic themes identified in respondents' quotes as reasons to raise equine welfare standards were ideological, protection of animal welfare, safe-guarding the reputation of the equine industry and safety (of people, horses and environment). Themes for reasons for low standards were societal norms, fiscal pressures, indolence, indifference and ignorance. Themes underpinning potential means for achieving meaningful change (solutions) were legislation, enforcement, education/ training, fiscal remedies, increasing awareness and a combination of these. Mechanisms aimed at raising standards must be based on an understanding of motivational drivers for currently low standards. The challenge is to translate the findings and this heightened awareness into meaningful change to the benefit of horses and those who care for them.

A case study of equine welfare on an Irish farm 2007-2009

Collins, J.[1], More, S.J.[1], Hanlon, A.[2], Duggan, V.[2]

[1] UCD CVERA, [2] UCD School of Agriculture, Food Science and Veterinary Medicine

Since 2007 there has been a growing concern for equine welfare in Ireland, in particular the issue of unwanted horses in a faltering economy. This report describes the progression in welfare standards for horses on a horse farm in Ireland during 2007, 2008 and 2009. Visits to the farm were undertaken and information, in the form of written notes and digital recording of observations and examinations, was gathered in consultation with officials from the Gardaí (the Irish police), the Department of Agriculture, Fisheries and Food (DAFF) and the Irish Society for the Prevention of Cruelty to Animals (ISPCA). Further independent veterinary corroboration of clinical findings and laboratory support occurred post seizure of horses. The complex reality of on-farm equine welfare problems and the difficulties in achieving a resolution are discussed compared to other species conventionally considered to be food-producing animals. This case report describes the conditions for horses on a commercial farm in Ireland during a period of growing concern for equine welfare, the inappropriate response (by the owners/keepers) to assistance and advice offered, and the largely futile though well-intentioned efforts made (by the relevant authorities) to address deficiencies until the effective abandonment of the animals forced the first ever seizure of horses for disposal and destruction under the terms of the European Communities (Welfare of Farmed Animals) Statutory Instrument no. 14 of 2008.

Development and application of a protocol to assess the welfare of equidae at fairs and markets in Ireland

Collins, J.[1], Johnson, J.[2], Hanlon, A.[2], More, S.J.[1], Duggan, V.[2]

[1] UCD CVERA, [2] UCD School of Agriculture, Food Science and Veterinary Medicine

Unregulated horse gatherings, such as fairs and markets, are considered to be of high value culturally, socially and economically to Ireland. However, a Delphi study completed in 2008 by Collins and others (2009; 2010 in press) identified welfare standards for horses at such events as one of the two most significant equine welfare concerns in Ireland. In this paper we describe the development of an equine welfare assessment protocol, based on the Five Freedoms, which employs measurement of both welfare inputs and outputs, and early attempts to apply this protocol at fairs and markets in Ireland. Thirty parameters (based on a semi-qualitative 5 point Likert scale) were each measured on thirty occasions alongside details of weather, horse numbers and the presence of an organising committee. The data set (n=30) was divided into three subsets (with some overlap) to enable the analyses of welfare scores for individual events (n=14), inter-observer variability in scores at events scored simultaneously but independently by the first two authors, JC and JJ (n=5), and change over time at any one event (n=8). By the criterion of importance adopted by the authors, the freedom of horses to access water, feed and shelter (resource input) and the ability of horses to drink and feed (current output) were deemed to be unduly compromised. The presence of an organising committee was not found to be protective of equine welfare. It is suggested by the authors that a combined approach (input and output-based measures) to the assessment of equine welfare at unregulated gatherings be adopted but that further work is required to refine the protocol to ensure that repeatability and reproducibility of scoring are achieved in its implementation.

The keeping and disposal of horses: an Irish perspective

Collins, J.[1], Hanlon, A.[2], More, S.J.[1], Wall, P.G.[3], Duggan, V.[2]

[1] UCD CVERA, [2] UCD School of Agriculture, Food Science and Veterinary Medicine, [3] UCD Geary Institute

Links between the structures, governance and funding of the Irish equine industries and potential concerns for equine welfare have already been reported (Collins *et al.,* 2008). The role of Local Authorities, social horse projects, the Farm Animal Welfare Advisory Council (FAWAC) and routes for the disposal of horses in relation to the keeping of horses in Ireland are further described in this paper with particular reference to equine health and welfare. Primary information was gathered through visits conducted to horse pounds, social horse projects, FAWAC and its Equine Welfare Working Group, horse dealer yards, ferry ports, horse slaughter plants and knackeries. Supplementary information was gathered through internet and telephone research. The contribution each makes to defining how horses are kept and disposed of in Ireland is described. Differences in the approach adopted between members of a given group (for example, different Local Authorities or different ferry ports) are highlighted. Inferences are drawn that should help to improve the landscape for all who aspire to keep horses with due regard to safeguarding their health and welfare. A fundamental issue remains the provision of a comprehensive, integrated system for the identification of equidae.

A qualitative approach to identifying solutions to selected equine welfare problems in Ireland

Collins, J.[1], Hanlon, A.[2], More, S.J.[1], Wall, P.G.[3], McKenzie, K.[3], Duggan, V.[2]

[1] UCD CVERA, [2] UCD School of Agriculture, Food Science and Veterinary Medicine, [3] UCD Geary Institute

This study was conducted to engage those who own, keep or manage horses or horse enterprises with efforts to safeguard equine welfare, and to provide informed perspective to those who are charged with the governance of equine health and welfare matters in Ireland. This paper aims to explore the views of those in industry and government regarding necessary improvements to equine welfare in Ireland at unregulated gatherings and during the disposal process. Qualitative research methods were employed, namely recorded semi-structured interviews, focus groups and a structured, facilitated workshop. Representatives from industry, welfare societies, socially disadvantaged social groupings and government engaged with the process, and shared their views regarding horse welfare priorities and solutions with merit to address welfare problems. A consensus was achieved that equine welfare in Ireland could be improved by the development of a comprehensive identification system, a Code of Practice for horse gatherings, a horse licensing scheme, a method to ensure that funds are ring-fenced to benefit humane horse disposal and improved means of raising awareness of the value of safeguarding horse welfare. The information gathered and the methods used have been and could be further employed to improve dialogue between the key players in industry and those in government charged with overseeing the sector to ensure practical evidence-based policies are produced to improve the welfare standard of horses in Ireland.

Development and application of a bio-security assessment protocol at equine events in Ireland

Johnson, J.[1], More, S.J.[2], Collins, J.[2], Duggan, V.[1]

[1] UCD School of Agriculture, Food Science and Veterinary Medicine, [2] UCD CVERA

There is the potential for disease transmission wherever horses gather in groups. The inconsistent application of disease control measures across the horse industries in Ireland, the highly contagious nature of many of the equine infectious diseases, the potential for the spread of disease from sub-clinically infected horses and increased transport of horses to international events together mean that all horses are put at risk of disease transmission, particularly in the event of an outbreak of an exotic equine disease. The objectives of this study were to develop a bio-security assessment tool for use at equine events, to apply the tool in the investigation of the potential for contagious disease transmission at equine events in Ireland, and to determine the influence of the degree of regulation of event on risk of disease transmission. A scoring system was developed to identify bio-security risks at equine events. This was based on both direct and indirect risk factors which contribute to contagious disease transmission. Risk factors included categories such as contact between horses, contact with fomites, feeding facilities, degree of public access, control of wildlife, sanitation of stables and housing ventilation. The regulation status of each event was determined based on a combination of published governing rules and observation of their enforcement during on-site visits. Highly regulated events had significantly lower overall risk levels than partially regulated events ($P<0.003$); overall risk level of partially regulated events did not differ significantly from that of unregulated events ($P=0.051$).

Bovine welfare

Beef farmers' perception of farm animal welfare

Dwane, A.[1], More, S.J.[2], McKenzie, K.[3], Blake, M.[4], Wall, P.G.[3], Hanlon, A.[1]

[1] UCD School of Agriculture, Food Science and Veterinary Medicine, [2] UCD CVERA, [3] UCD Geary Institute, [4] Department of Agriculture, Fisheries and Food

The role of farmers and other stakeholders in developing and implementing policy on farm animal welfare is likely to underpin the success of such initiatives. This ongoing research explores the perceptions of beef farmers to animal welfare and mechanisms to further improve on-farm welfare, using the 'Animal Welfare, Recording, and Breeding (AWRB) Scheme for Suckler Herds' as a case study.

Refining current systems of early warning and prevention of on-farm animal welfare incidents

Kelly, P.[1], McKenzie, K.[2] More, S.J.[3], Blake, M.[1], Hanlon, A.[4]

[1] Department of Agriculture, Fisheries and Food, [2] UCD Geary Institute, [3] UCD CVERA, [4] UCD School of Agriculture, Food Science and Veterinary Medicine

In 2004, the then Minister for Agriculture and Food in Ireland announced that he had accepted the recommendation of the Irish Farm Animal Welfare Advisory Council for the establishment of a collaborative, nationwide early warning / intervention system for farm animal welfare cases. The Early Warning System currently involves a partnership between the Department of Agriculture, Fisheries and Food, the Irish Farmers Association and the Irish Society for the Prevention of Cruelty to Animals that aims to identify and address real or potential cases in which the welfare of farm animals is compromised. This study seeks to better identify and understand risk factors associated with on-farm animal welfare incidents in Ireland and thereby provide opportunities to refine current systems of early warning and prevention.

Cadmium exposure in cattle

Cadmium exposure in cattle: a review

Lane, E.A.[1], Canty, M.J.[1]

[1] UCD CVERA

No biological role has been described for cadmium (Cd) in animals and its presence in animal tissue is considered unnecessary. Cadmium is considered to be one of the most toxic substances in the environment due to its wide range of organ toxicity and long elimination half-life. Batteries are an important source of Cd pollution, additionally, combustion of coal, smelting, mining, alloy processing and industries that employ Cd as a dye are also potential sources of Cd pollution. Agricultural practices such as the application of sewage sludge and contaminated fertilizers are also sources of Cd contamination. Absorption of Cd occurs via the respiratory and digestive system. Approximately 10 to 50% of Cd fumes are absorbed by the respiratory system. While, Cd is poorly absorbed via the digestive tract, compared to similar divalent cations, Zn and Fe; approximately 5% of oral Cd is absorbed. Once absorbed, Cd circulates in red blood cells or bound to albumin in plasma. Cadmium interacts with the metabolism of essential minerals; calcium, zinc, iron, copper and selenium. The majority of newborn ruminants have a low Cd burden. Accumulation occurs slowly over time, primarily in liver and kidneys. In the liver it may induce and bind metallothionein, this complex is released slowly into circulation and then accumulates in kidneys. At high levels dietary Cd can cause decreased feed intake, and lowered weight gain, anaemia, decreased bone absorption and abortions and Cd toxicity has been reported in many species including cattle. This paper reviews the literature pertaining to Cd exposure and its effects in cattle.

Teaching methods

Problem-based learning in veterinary education

Lane, E.A.[1]

[1] UCD CVERA

Problem-based learning (PBL) replicates life experiences to stimulate learning, the integration of knowledge, and lifelong learning skills, all of which are requirements for veterinary medical education. As the curricular content of veterinary schools expands to immense proportions following advances in medical knowledge and biotechnology, it becomes impracticable to ensure that all students at the beginning of their careers have such a wide knowledge base. Students who are faced with vast amounts of information to learn by rote, much of which may seem irrelevant to their prospective career, may become disillusioned with their chosen course, hence the temptation to convert to a PBL curriculum. The PBL strategy of teaching is becoming increasingly popular in veterinary faculties worldwide, encompassing both curriculum content and a process of learning. In PBL, clinical cases are carefully selected to provoke deep student learning by the acquisition of both basic scientific and clinical knowledge critical to the case; cultivate problem-solving abilities; and encourage the development of team-building, self-directed learning, communication, and self- and peer-assessment skills. Problem-solving skills, understanding of the basic sciences, and clinical performance are all improved by the PBL process. The aim of this paper is to review a decade of literature pertaining to the inclusion of PBL in veterinary and medical curricula.

Methodological issues

Defining output-based standards to achieve and maintain tuberculosis freedom in farmed deer, with reference to member states of the European Union

More, S.J.[1], Cameron, A.R.[2], Greiner, M.[3], Clifton-Hadley, R.S.[4], Correia Rodeia, S.[5], Bakker, D.[6], Salman, M.D.[7], Sharp, J.M.[8], De Massis, F.[5], Aranaz, A.[9], Boniotti, M.B.[10], Gaffuri, A.[11], Have, P.[5], Verloo, D.[5], Woodford, M.[12], Wierup, M.[13]

[1] UCD CVERA, [2] AusVet Animal Health Services, Cuiseaux, France, [3] Federal Institute for Risk Assessment (BfR), Berlin, Germany, [4] Veterinary Laboratories Agency, Weybridge, Surrey, England, [5] European Food Safety Authority, Parma, Italy, [6] Department of Bacteriology and TSEs, Central Institute for Animal Disease Control, Lelystad, The Netherlands, [7] Animal Population Health Institute, College of Veterinary Medicine and Biomedical Sciences, Colorado State University, Colorado, USA, [8] Veterinary Laboratories Agency, Midlothian, Scotland, [9] Departamento de Sanidad Animal, Facultad de Veterinaria, Universidad Complutense, Madrid, Spain, [10] Istituto Zooprofilattico Sperimentale della Lombardia e dell'Emilia Romagna, Dipartimento di Biologia Molecolare, Brescia, Italy, [11] Istituto Zooprofilattico Sperimentale della Lombardia e dell'Emilia Romagna, Sezione Diagnostica di Bergamo, Bergamo, Italy, [12] Algarve, Portugal, [13] Department of Biomedical Sciences and Veterinary Public Health, Faculty of Veterinary Medicine and Animal Sciences, Swedish University of Agricultural Sciences, Sweden

Within the European Union (EU), detailed legislation has been developed for cattle, but not deer, to minimise disease risks associated with trade in animals and animal products. This legislation is expressed as input-based standards, providing a detailed outline of the activity required (for example, testing of animals and application of defined control measures), on the expectation that an adequate output (for example, confidence in freedom) will be achieved. Input-based standards are at odds with the increasing shift towards output-based standards, particularly in OIE rules governing international trade. In this paper, we define output-based standards to achieve and maintain freedom from tuberculosis (TB) in farmed deer, with reference to EU member states. After considering the probability of freedom achieved for cattle under existing EU legislation, we defined a 'free farmed deer holding' as one with a probability of freedom from infection of at least 99%. We then developed an epidemiological model of TB surveillance systems for deer holdings, incorporating different surveillance strategies, including combinations of diagnostic tests, and a variety of different scenarios relating to the potential for introduction of infection. A range of surveillance strategies were identified to achieve and maintain a free farmed deer holding, and worked examples are presented. The surveillance system sensitivity for varying combinations of screening and confirmatory tests in live animals, animals at slaughter and on-farm deaths is also presented. Using a single test at a single point in time, none of the TB tests routinely used in farmed deer is able to achieve an acceptable probability of TB freedom. If repeat testing were undertaken, an acceptable probability of TB freedom could be achieved, with differing combinations of the surveillance system sensitivity, frequency of testing and risk of introduction. The probability of introduction of infection through the importation of infected deer was influenced by the use of a pre-movement test (assumed 90% test sensitivity and negative test results), the TB prevalence in the source herd and the number of animals imported. A surveillance system sensitivity of at least 81% was achieved with different combinations of annual live animal surveillance and surveillance of animals at slaughter or on-farm deaths. This methodology has broad applicability and could also be extended to other diseases in both deer and other species with relevance to trade in animals and animal products.

The use of Geographic Information System (GIS) and non-GIS methods to assess the external validity of samples post-collection

Richardson, E.[1], Good, M.[2], McGrath, G.[3], More, S.J.[3]

[1] Teagasc Moorepark Dairy Production Research Centre, [2] Department of Agriculture, Fisheries and Food, [3] UCD CVERA

External validity is fundamental to veterinary diagnostic investigation, reflecting the accuracy with which sample results can be extrapolated to a broader population of interest. Probability sampling methods are routinely used during the collection of samples from populations, specifically to maximize external validity. Non-probability sampling (e.g., of blood samples collected as part of routine surveillance programs or laboratory submissions) may provide useful data for further *post hoc* epidemiological analysis, adding value to the collection and submission of samples. As the sample has already been submitted, the analyst or investigator does not have any control over the sampling methodology, and hence external validity as routine probability sampling methods may not have been employed. The current study describes several Geographic Information System (GIS) and non-GIS methods, applied *post hoc,* to assess the external validity of samples collected using both probability and non-probability sampling methods. These methods could equally be employed for inspecting other datasets. Mapping was conducted using ArcView 9.1. Based on this *post hoc* assessment, results from the random field sample could provide an externally valid, albeit relatively imprecise, estimate of national disease prevalence, of disease prevalence in 3 of the 4 provinces (all but Ulster, in the north and northwest, where sample size was small), and in beef and dairy herds. This study provides practical methods for examining the external validity of samples post-collection.

Improving the quality of reporting in veterinary journals: how far do we need to go with reporting guidelines?

More, S.J.[1]

[1] UCD CVERA

Publication in the international peer-reviewed literature is one of the most important outputs of any research, providing a public record of research conducted. However, the quality of reporting is variable, both in the medical and veterinary literature. In response to these concerns, a number of guidelines have been developed by international scientific teams to promote the quality of reporting of research studies. These guidelines are written as checklists, flow diagrams, or in the form of explicit text, specifying the minimum information that is required in each section of a published paper to provide a transparent, accurate and complete account of the research. Increasingly, key medical journals either require or recommend author compliance with the above-mentioned reporting guidelines. As yet, however, a similar approach is not standard practice among veterinary journals. In this Personal View, it is argued that veterinary journals should require author compliance with relevant reporting guidelines, in the interest of high quality reporting of veterinary medical research.

International collaboration

Avian influenza

An outbreak of highly pathogenic avian influenza at a public animal exhibit in Seoul, Korea, during 2008

Yoon, H.[1], Moon, O.-K.[1], More, S.J.[2], Park, C.-K.[1], Park, J.-Y.[1], Lee, Y.-J.[1], Lee, S.-D.[1], Ha, J.-K.[1], Jeong, S.-K.[1], Jeong, J.-W.[1], Lee, S.-J.[1]

[1] National Veterinary Research and Quarantine Service, Republic of Korea, [2] UCD CVERA

This study describes the first recorded outbreak of HPAI in the city of Seoul, in captive birds held in an exhibition for public viewing at a local district office. The index cases were two pheasants, which had been introduced into the exhibit on 24 April, 4 days prior to death, from a store in a local market in Gyeonggi-do. Ducks and chickens from an HPAI outbreak farm, subsequently confirmed on 4 May, had also been held in this store. This outbreak highlights the potential role of local markets in AIV transmission. This outbreak led to considerable public health concern in Korea, however, no human cases were reported. The non-commercial poultry sector needs to be considered in national plans for preparedness and response.

Brucellosis

Surveillance and control of bovine brucellosis in the Republic of Korea during 2000–2006

Lee, B.-Y.[1], Higgins, I.M.[2], Moon, O.-K.[1], Clegg, T.A.[2], McGrath, G.[2], Collins, D.M.[2], Park, J.-Y.[1], Yoon, H.-C.[1], Lee, S.-J.[1], More, S.J.[2]

[1] National Veterinary Research and Quarantine Service, Republic of Korea, [2] UCD CVERA

Bovine brucellosis is a major animal health problem in the Republic of Korea. Further, a number of human cases of brucellosis have recently been detected. This paper provides an overview of surveillance (to detect new cases) and control (to clear infection following case detection) of bovine brucellosis in the Republic of Korea during 2000–2006. Using data from AIMS (the national animal infectious disease data management system), we conducted separate descriptive analyses, initially using farm and then episode as our unit of interest. An episode was defined as a period of compulsory herd trading restriction, following detection of infection with *Brucella abortus* in one or more cattle. We also identified risk factors for two measures of disease control: episode duration (logistic generalised estimating equation model) and time to re-restriction (Cox's proportional hazard model). There were 8530 and 52,739 reactor farms and reactor cattle, respectively, during 2000–2006. From 2004 to 2006, there was a substantial increase in the number of new outbreaks, particularly within the beef sector. The probability of a prolonged episode (>150 days) and the hazard of a second episode each increased with herd size. Further, the hazard of a second episode was higher in 2005 (compared with other years) and in the southeast of Korea (compared with other provinces). The effect of outbreak size on control varied between the beef and dairy sectors. The increase in beef cattle reactors in 2004–2006 is closely aligned to an increase in surveillance effort. Nonetheless, it is likely that this is a genuine reflection of the recent establishment and spread of

Characteristics of bovine brucellosis on Korea during 2001-2004

Nam, H.-M.[1], Kim, C.-H.[2], More, S.J.[3], Yoon, H.[1], Kim, S.-J.[1], Lee, B.-Y.[1], Park, C.-K.[1], Jeon, J.-M.[1], Wee, S.-H.[1]

[1] National Veterinary Research and Quarantine Service, Republic of Korea, [2] Ministry for Food, Agriculture, Forestry and Fisheries, Republic of Korea, [3] UCD CVERA

This paper describes the epidemiological characteristics of bovine brucellosis in Korea during January 2001-September 2004, which encompasses the period when the incidence of bovine brucellosis increased abruptly. Data from the national Animal Infectious Disease Data Management System were used for this study. A range of epidemiological measures were calculated including annual herd and animal incidence. During the study period, there were 1,183 outbreaks on 638 farms. Annual herd incidence in beef cattle increased from 0.12 to 11.5 outbreaks per 10,000 from 2001 to 2004 (to September), respectively. On 401 (62.9%) farms during this period, infection was eradicated without recurrence. Recurrence of infection was significantly higher on farms where abortion was reported (53.3%) compared to farms where it was (30.0%). On beef cattle farms, infection was introduced most frequently through purchased cattle (46.2%). Based on the results of this study, the recent establishment and spread of brucellosis in the Korean beef cattle population was mainly due to incomplete or inappropriate treatment of aborted material and the movement of infected cattle. This study has provided scientific data to underpin ongoing revision of national disease control regulations, specifically an extension to the period of movement restriction and of the retest interval in test-positive herds.

Foot and mouth disease

Using field-based epidemiological methods to investigate FMD outbreaks: an example from the 2002 outbreak in Korea

Wee, S.-H.[1], Nam, H.-M.[1], Moon, O.-K.[1], Yoon, H.[1], Park, J.-Y.[1], More, S.J.[2]

[1] National Veterinary Research and Quarantine Service, Republic of Korea, [2] UCD CVERA

Relevant to foot and mouth disease (FMD), most published epidemiological studies have been conducted using quantitative methods and substantial regional or national datasets. Veterinary epidemiology also plays a critical role during outbreak investigations, both to assist with herd-level decision-making and to contribute relevant information to assist with ongoing national or regional control strategies. Despite the importance of this role, however, little information has been published on the use of applied (field-based) epidemiological methods during disease outbreaks. In this study, we outline an investigative template for FMD, and a case study of its use during the 2002 FMD outbreak in Korea. Suitable for use during field-based epidemiological investigations of individual farms within a broader regional/national response, the template considers three steps including confirming infection, estimating date of introduction and determining

method of introduction. A case study was conducted on IP13 (the 13th infected premises), the only IP during the 2002 FMD outbreak in Korea that was geographically isolated from all other known cases. The authorities first became aware of FMD on IP13 on 2 June, however, infection may have been present from 12 May. Infection was confirmed on 3 June 2002. FMD was probably spread to IP13 by a contract worker who had participated during 2–4 May in the culling operations on IP1. Other routes of spread were ruled out during the investigation. The contract worker lived in the locality of IP13 and worked on a part-time basis at a pork-processing plant that was adjacent to this farm. The contractor became heavily contaminated during the cull, but did not comply fully with cleaning and disinfection requirements once the cull had been completed. The investigative template contributed structure and focus to the field-based investigation. Results from this case study demonstrate the need for strict management of personnel in disease control and adherence to the sanitary rules by all those involved.

Epidemiological characteristics of the 2002 outbreak of foot-and-mouth disease in the Republic of Korea

Wee, S.-H.[1], Yoon, H.[1], More, S.J.[2], Nam, H.-M.[1], Moon, O.-K.[1], Jung, J.-M.[1], Kim, S.-J.[1], Kim, C.-H.[1], Lee, E.-S.[1], Park, C.-K.[1], Hwang, I.-J.[1]

[1] National Veterinary Research and Quarantine Service, Republic of Korea, [2] UCD CVERA

The Republic of Korea experienced a foot-and-mouth disease (FMD) outbreak during May–June 2002. The present study describes epidemiological characteristics of the 2002 FMD outbreak in Korea, including the pattern of the outbreak in both time and space, transmission routes among infected farms, and control measures. One of the notable features of the 2002 FMD epidemic in Korea was that the virus infected mostly pigs [15 of 16 infected premises (IPs)], despite the presence of other susceptible animals on infected and neighbouring farms. The epidemic showed temporal clustering at 8–9 day intervals, suggesting five generations of infection during the outbreak, and 13 of 16 (81.3%) IPs were located within a 10 km-radius of the index case. The clinical signs that prompted notification of infection included vesicles around hooves and snouts. The age of lesions was significantly less among cases reported by farmers compared with veterinarians. The high awareness of farmers from an earlier FMD outbreak greatly helped the animal hygiene authority in efforts associated with disease control and eradication. The outbreak was eradicated within < 2 months as a result of the intensive control efforts of the animal hygiene authorities and the cooperation of the Korean people. Although the outbreak was a costly lesson for the Korean people, the experience gained will contribute to future efforts in the prevention and control of animal infectious diseases.

OTHER ANIMAL HEALTH ISSUES
International collaboration

Tuberculosis

Mycobacterium bovis in Korea: an update

Wee, S.-H.[1], Kim, C.-H.[2], More, S.J.[3], Nam, H.-M.[1]

[1] National Veterinary Research and Quarantine Service, Republic of Korea, [2] Ministry for Food, Agriculture, Forestry and Fisheries, Republic of Korea, [3] UCD CVERA

This paper reports changes in the cattle population and the incidence of bovine tuberculosis in the Republic of Korea between 1960 and 2007, and discusses potential factors contributing to the recently observed increase in disease incidence, particularly in beef cattle and deer. Although there have been ongoing refinements to the existing programme, further improvements in current strategies are needed, including surveillance of susceptible animal species, both domestic and wild, and ongoing surveillance of the human population.

Miscellaneous

Toxoplasma gondii in meat and meat products: detection & risk assessment

Halova, D.[1], Zintl, A.[1], McCarthy, E.[1], Mulcahy, G.[1], Murphy, T.[1], Rafter, P.[2], Collins, D.M.[3], De Waal, T.[1]

[1] UCD School of Agriculture, Food Science and Veterinary Medicine, [2] Department of Agriculture, Fisheries and Food, [3] UCD CVERA

Toxoplasma gondii is a protozoan parasite that can infect virtually all warm-blooded animals including man. Humans can be infected by handling or consuming raw or undercooked meat from infected animals, or food contaminated with cat faeces. Between 37 and 58% of women of childbearing age are seropositive for Toxoplasma in various European countries. Very little data is available in Ireland on the prevalence of *Toxoplasma gondii* in meat and meat products. In order to fill these gaps, a cross-sectional abattoir study was undertaken to determine the prevalence of *T. gondii* in food animals in Ireland. Results to date indicate that 35.5% of sheep, 4.7% of pig, 6.6% of deer and 1% of chicken samples tested positive for *T. gondii* using the Latex agglutination test.
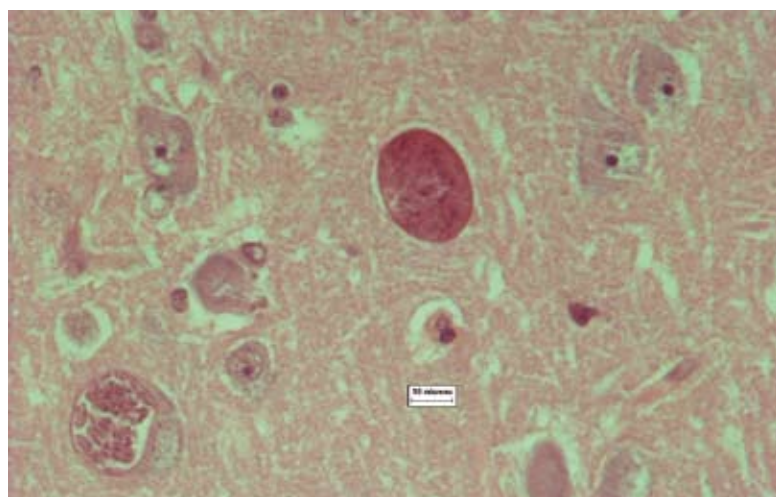
Tissue cyst of Toxoplasma gondii in brain of a sheep. Photograph by T. de Waal.

General support

GENERAL SUPPORT

Epidemiological support

Key CVERA contacts: Simon J. More, Liz Lane, Inma Aznar, Mary Canty

Farm investigations

Farm investigations are a critical component of CVERA's work. These investigations offer the opportunity for CVERA staff to support veterinary students in the use of practical epidemiological skills to solve (often complex) on-farm problems. Key epidemiological skills concern the use of simple methodologies to examine patterns of disease presentation in time, in space and among different animal groupings. Farm investigations, which are a key component of the final year curriculum, are conducted in collaboration with local private veterinary practitioners and/or veterinary inspectors. The following investigations were conducted during 2008 and 2009:

- Mastitis (Co. Meath, January 2008)
- General calf health (Co. Meath, February 2008)
- Abortion (Co. Wicklow, February 2008)
- Mastitis (Co. Meath, February 2008)
- Fertility (Co. Waterford, March 2008)
- Calf pneumonia (Co. Dublin, April 2008)
- Calf mortality (Co. Tipperary, May 2008)
- General goat health (Co. Meath, August 2008)
- Tuberculosis (Co. Wicklow, October 2008)
- Tuberculosis (Co. Monaghan, November 2008)
- Calf mortality (Co. Wicklow, November 2008)
- Tuberculosis (Co. Louth, November 2008)
- General calf health (Co. Limerick, December 2009)

International collaboration

CVERA provides ongoing epidemiological support in a range of countries, including:

- *Chile.* Technical support to the Servicio Agrícola y Ganadero [Agriculture and Livestock Service] in their work towards the establishment of a national eradication programme for bovine tuberculosis.

- *Korea.* Technical support to the epidemiology unit at the National Veterinary Research and Quarantine Service in their ongoing epidemiological work, in support of national decision-making, on foot and mouth disease, bovine brucellosis, bovine tuberculosis and avian influenza.

- *Kyrgyzstan.* Technical support to the EU Budget Support Programme in Kyrgyzstan and the Kyrgyz State Veterinary Department (SVD) towards the establishment of an epidemiological unit within SVD.

Epidemiological training

CVERA provides both formal and informal epidemiological support to veterinarians in Ireland.

A course in introductory epidemiology (so-called 'Demystifying Epidemiology') was held on three occasions during 2008 and 2009:

- Tullamore, Co. Offaly (with an emphasis on veterinary public health, 12 May 2008),
- Thurles, Co. Tipperary (for field veterinarians, 13 May 2008)
- Backweston campus, Co. Kildare (for laboratory and policy veterinarians, 14 May 2008), and
- Drumshanbo, Co. Leitrim (for field veterinarians, 15 May 2008).

The following provides a background to this course:

'Epidemiology is often viewed as a discipline of facts and figures, with only limited application to front-line veterinarians on the ground. The purpose of this one-and-a-half day course is to demystify epidemiology, and provide attendees with a sound understanding of epidemiology in action. The course is problem-based, and will centre on a range of hands-on learning exercises that are relevant to DAFF veterinarians in the field. Following this course, there will be an opportunity for interested attendees to join a mentored study group that will meet on an ongoing basis.'

An epidemiological mentoring group continues to meet on a six-monthly basis, to support DAFF veterinarians with an interest in the practical application of veterinary epidemiology in their work. The group met in Portlaoise, Co. Laois, in May and November 2008, and in May and November 2009. The meeting includes a session of formal epidemiology training (for example, 'writing scientific papers', epidemiological study designs' etc). In addition, a group of epidemiological mentors are providing support for the epidemiological project that each member is conducted.

An outline of some of the current projects is included overleaf.

Summer grazing pastures, eastern Kyrgyzstan. Photograph by S.J. More.

A review of bovine cases consigned under veterinary certification to emergency and casualty slaughter in Ireland during 2006 to 2008

Mary Cullinane[1], Edmond O'Sullivan[2], Gerald Collins[1] [Mentors: Daniel M. Collins[3], Simon J. More[3]]

[1] Department of Agriculture, Fisheries and Food, [2] Cork County Council, [3] UCD CVERA

In Ireland, a small number of bovine animals whose health or fitness for transport is in doubt, may, subject to a veterinary certificate, be presented for slaughter for human consumption either as an emergency or casualty slaughter animal. The transport of these animals must be done such that their welfare is not compromised at any stage from farm to slaughter. As yet, however, there is little published research on this issue. The objective of this study is to review bovine cases consigned under veterinary certification to emergency or casualty slaughter in Ireland during 2006 to 2008.

Examining the relationship between the gamma interferon test and the single intradermal comparative tuberculin test (SICTT) in South Tipperary

Martin Hayes[1, 2] [Mentors: Inma Aznar[2], Simon J. More[2]]

[1] Department of Agriculture, Fisheries and Food, [2] UCD CVERA

The TB project in South Tipperary is examining the correlation between the Gamma Interferon test and the single intra-dermal comparative tuberculin test (SICTT) in a percentage of reactors disclosed in a 12 month period commencing June 2009. It is hoped to use these data to assist in interpretation of animals in the future.

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

Mary Bourke[1] [Mentors: Inma Aznar[2], Simon J. More[2]]

[1] Department of Agriculture, Fisheries and Food, [2] UCD CVERA

The purpose of this project is to investigate the possible pseudo-vertical transmission of tuberculosis between a dam and her suckling offspring via infected milk. The role of *Mycobacterium bovis* infected milk in passing on infection to dairy calves has been recognised in a study by Renteria Evangelista and Hemandez De Anda (1996) and in a more recent study carried out by Doran *et al.,* (2009). These studies demonstrated that a cow who is shedding *Mycobacterium bovis* in her milk is capable of infecting calves being fed on this milk. It stands to reason therefore that a suckler cow if infected with tuberculosis could be a source of infection for her calf.

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

Nicky Fennelly[1] [Mentors: John Griffin[1], Tracy A. Clegg[2], Guy McGrath[2], Simon J. More[2]]

[1] Department of Agriculture, Fisheries and Food, [2] UCD CVERA

The use of epidemiological methods is an intrinsic component of disease management and control. The objective of this study is to examine Bovine Tuberculosis (BTB) in County Kilkenny cattle herds from 1998 to 2008 inclusive

and use the information to enhance the understanding of the epidemiology of the disease in the county. The study will examine the long term changes that have occurred over the decade (secular trends), and will investigate cyclical, seasonal and spatial disease patterns.

Factors contributing to the sample quality for the BSE active surveillance programme

Aidan Cahill[1] [Mentors: Daniel M. Collins[2], Simon J. More[2], Hazel Sheridan[1]]

[1] Department of Agriculture, Fisheries and Food, [2] UCD CVERA

Ireland's active surveillance programme for BSE involves the collection of brain stem tissue samples from certain categories of cattle, including cattle which die on farm (fallen cattle). However, some of these samples are of poor quality due to autolysis. This has implications for BSE diagnosis. Ireland is obliged under EU law to take measures to minimise sample autolysis. Data on the degree of autolysis of all samples in 2007, 2008 and 2009 have been captured on the AHCS (Animal Health Computer System). Date of test combined with mapping of farm of origin can be used to infer temporal and spatial patterns in this body of data. The objective of this study is to identify risk factors for poor sample quality in fallen animals in the Republic of Ireland, with a view to taking corrective action to improve overall sample quality.

Irish BSE cases born after October, 2006: descriptive epidemiology and spatial analysis

Eoin Ryan[1] [Mentors: Guy McGrath[2], Daniel M. Collins[2], Inma Aznar[2], Simon J. More[2], Hazel Sheridan[3]]

[1] Central Veterinary Research Laboratory, Department of Agriculture, [2] UCD CVERA, [3] Fisheries and Food, Department of Agriculture, Fisheries and Food

Cases of BSE continue to occur in Irish cattle born after the reinforced feed ban (BARB) and enhanced meat and bone meal controls established in October 1996. The epidemiology of these cases is to be investigated and described in order to inform aetiological hypotheses. Spatial analysis will be carried out to determine whether the distribution of these cases is non-random and the factors that may relate to their distribution.

€uromilk – a team-based approach to milk quality and mastitis control on Irish dairy farms

Finola McCoy[1], Catherine Devitt[2] [Mentor: Simon J. More[3]]

[1] Teagasc Moorepark Dairy Production Research Centre, [2] Research consultant, [3] UCD CVERA

There is a wealth of knowledge available in the dairy industry in relation to mastitis prevention and control. Many countries have used this information to develop national mastitis control programmes, however no such structure exists in the Irish dairy industry. Work from the USA has shown that the formation of milk quality teams was successful in encouraging farms to adopt recommended management practices and improve communication between producers and dairy professionals. This pilot study, involving 23 Irish farms will look at the impact and feasibility of a team-based approach to milk quality. It also aims to identify recommendations and requirements for future programme development and delivery.

Statistical support

Key CVERA contact: Tracy A. Clegg

Statistical support and advice

During 2008-2009, in addition to core projects, CVERA provided statistical support and advice to a range of researchers. Projects currently underway include:

Department of Agriculture, Fisheries and Food
- Examining whether herds that buy in during a restriction due to *M. bovis* are at increased risk of a prolonged episode or subsequent episode of *M. bovis*
- Epidemiological studies of poor animal performance on a farm in Castlecomer, Co. Kilkenny
- Serological surveillance of cattle for bluetongue in Ireland
- Descriptive investigation of animals slaughtered under a veterinary certificate during 2006-2008

UCD School of Agriculture, Food Science and Veterinary Medicine
- Use of pooled serum to predict herd prevalence of BVD and IBR
- A critical evaluation of farm-level milk quality, based on milk recording data
- Persistent-post breeding endometritis in mares
- UCD review of horse welfare in Ireland 2007-2009
- Comparison of the Immulite® and RIA assay methods for measuring peripheral blood P4 levels in Greyhound bitches prior to breeding

Geographic Information Systems (GIS) support

Key CVERA contacts: Guy McGrath, Daniel M. Collins

The Wildlife Unit

a. An independent monitor

CVERA acts as an independent monitor for the National Parks and Wildlife Services (the Department of the Environment, Heritage and Local Government) to ensure operations of the Wildlife Unit (DAFF) are within pre-agreed criteria. This includes verifying individual badger removal licences and maintaining checks on areas treated by the Wildlife Unit on a county by county basis through time. Ongoing reports with thematic maps are produced for the two government Departments.

b. Administration

In addition to monitoring and reporting on Wildlife Unit activities, CVERA maintain the GIS component of the Wildlife Unit administration centre in Johnstown Castle, Co. Wexford. This centre provides all District Veterinary Offices with the relevant maps and ortho-photography to complete badger surveys in areas where tuberculosis breakdowns in cattle have been attributed to wildlife. The badger setts found through surveying are then digitised and maintained centrally on the GIS.

General mapping support

CVERA provide a broad range of mapping support, including:
- Maps for specific field investigations
- Maps for illustrative purposes in publications and internal reports
- Maps for aiding in the spatial aspects of study design
- Mapping to assist District Veterinary Offices
- Annual production of thematic prevalence maps for tuberculosis, brucellosis and BSE
- Provision of mapping assistance in the event of an emergency disease incursion.

Atmospheric dispersion modelling

CVERA have the capacity to perform atmospheric dispersion and deposition modeling. The software utilised is currently BREEZE AERMOD v7.0.58, BREEZE AERMET 6.2.0 and BREEZE 3D Analyst 2.0.56 Pro Plus versions © Trinity Consultants as used by the EPA.

Database support

The TB testing database

Key CVERA contacts: Isabella Higgins, Paul White

Introduction

The CVERA National Bovine Tuberculosis/Brucellosis testing database aims to provide an ancillary platform for storing TB/BR scheme data, and for running epidemiological queries. By maintaining a continuous record scheme data since 1989, and allowing this to be combined with data from other sources, the system provides a dynamic framework for data management within CVERA.

The database stores information on the tuberculosis/brucellosis eradication schemes in relation to:
- TB test summary data
- TB reactor and inconclusive skin results
- TB clear animal test results for restricted herds
- TB post-mortem results for reactor animals
- Laboratory results for animals submitted for TB Histopathology/Culture
- Contiguous herds identified by DAFF field staff
- Brucellosis test summary data

Development

Since its inception in 1998, the National Bovine Tuberculosis/Brucellosis testing database has undergone continuous development in response to the evolving data handling requirements of the research program. The database was proto-typed within Microsoft Access™ and data was originally drawn by means of diskette from the Nixdorf system for each DVO and then merged into a centralized database. With the advent of AHCS in 2005, the access database was migrated to Microsoft SQL Server™ as a platform capable of handling the increasing volume of data. Within SQL server, the database is now optimized for the efficient running of queries on large tables such as the TB test summary table (4.8 million records) and associated TB animal table (15.6 million records).

Management

Monthly updates from AHCS are obtained by running a series of standardized reports on AHCS and outputting to CSV text format. The text files are initially loaded into staging tables on the CVERA database via DTS packages. A validation/cleaning step is performed to ensure data consistency with AHCS, before final loading of data. This is followed by data manipulation as follows:
- coalescing of multiple part-test summary records into single records
- classification of reactors at test summary level according to size of skin test reaction (as standard/non-standard reactor)
- summarization of test summary data over the period 1989 to date as an episode file with one record describing each period of restriction
- summarization of herds in terms of number and severity of TB episode(s) to date

Interrogation

By means of Structured Query Language (SQL) queries, data stored across various tables may be summarized/combined. More recently, advances in PC hardware/software technology have enabled the running of more resource intensive queries, including questions relating animal movement/birth registration data to TB at animal/herd level.

General database maintenance and interrogation

Key CVERA contact: Isabella Higgins

To assist with a range of research projects, the following national databases are regularly interrogated:

- Animal Health Computer System (AHCS) database
- Animal Identification and Movement System (AIM) database
- Factory surveillance database
- Laboratory Information Management System (LIMS) database
- Tracing Onward Tracking System (TOTS)
- ER76 database
- Badger post mortem database (1997-2003)

The following examples illustrate how these data are subsequently used:

a. The provision of data for ongoing work, PhD theses and various papers, including:
   - Validation of Breeze Air Dispersion Modelling for the period 2000 to 2007 for a small area study
   - The genetics of predisposition to tuberculosis in Irish dairy and beef cattle
   - A range of projects relating to tuberculin registration
   - APT figures on a DED basis for production of thematic maps

b. Detailed work was conducted in collaboration with David Williams, formerly UCD Statistics, to summarise the TB test records for the period 1989-2009 and to create a suite of programs that will identify breakdown episodes for each herd on a national basis.

c. Provision of technical support and data for the production of improved statistical measures for TB surveillance and control. Initiated within CVERA, the project is in collaboration with David Williams formerly UCD Statistics.

d. National SICTT records between November 2000 and December 2007 were provided and summarized on an episode basis for the study Genetic associations between Johne's disease and susceptibility to *Mycobacterium bovis* and *Mycobacterium avium* subsp. *avium* in Irish Holstein Friesian dairy cows. This work was conducted by Máiréad Bermingham, formerly Teagasc Moorepark.

e. Provision of demographic data on 40 farms for the year 2005 for the study on prevalence and risk factors associated with *Cryptosporidium* infection. This data was requested by Valerie De Waele (Central Veterinary Research Laboratory, Backweston).

f. Data relating to tuberculin tests carried out on cattle, and the number of tuberculin reactors disclosed, according to county were compiled to produce thematic maps and the Bovine Tuberculosis Statistics, Annual Summary (2008-2009).

g. Provision of demographic data on farms selected for the study on molecular epidemiology of *Cryptosporidium parvum* subtypes using multi-locus subtyping approach and geographic information system approach. This data was requested by Valerie De Waele (Central Veterinary Research Laboratory, Backweston).

GENERAL SUPPORT
Database support

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**   *The Central Veterinary Research Laboratory (CVRL)*
***   *The Department of Zoology, Ecology & Plant Science, University College Cork*
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* *The TB Diagnostics and Immunology Research Centre and The Badger Vaccine Project*
** *The Central Veterinary Research Laboratory (CVRL)*
*** *The Department of Zoology, Ecology & Plant Science, University College Cork*
**** *The School of Natural Sciences, Trinity College Dublin*

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Veterinary Sciences Centre

UCD School of Agriculture, Food Science and Veterinary Medicine
University College Dublin, Belfield, Dublin 4, Ireland