# **Biennial Report**, 2004-05

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





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

ISBN: 1905 254 113





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# Biennial Report, 2004-05

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

S.J. More (editor)

ISBN :1905 254 113

### Preface

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

- 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 in support of DAF inspectorate and research staff in the area of animal health.

The TB Diagnostics and Immunology Research Centre and the Badger Vaccine Project each 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, including bovine tuberculosis, bovine brucellosis, transmissible spongiform encephalopathies, herd health and avian influenza. In addition, CVERA provides general support to government, industry and the veterinary profession (post pre- and post-graduation) on these and other animal health issues.

Simon More Centre for Veterinary Epidemiology and Risk Analysis

Eamonn Gormley The TB Diagnostics and Immunology Research Centre and The Badger Vaccine Project

Ligh b bern

Leigh Corner The Badger Vaccine Project

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### Acknowledgements

### The Centre for Veterinary Epidemiology and Risk Analysis

CVERA work closely with a wide range of organisations, both in Ireland and internationally. The collaborative input of staff from each of these organisations is gratefully acknowledged, including:

- In Ireland DAF (veterinary inspectorate and research staff central, regional, local), UCD School of Agriculture, Food Science and Veterinary Medicine, UCD School of Mathematics, the Irish Cattle Breeders Federation (ICBF), Veterinary Ireland and individual private veterinary practitioners, BirdWatch Ireland, the National Parks and Wildlife Service (within the Department of Environment, Heritage and Local Government), Teagasc, University College Cork, the Irish Equine Centre, the Marine Institute, a wide range of industry organisations, and individual Irish farmers
- In Canada the University of Guelph
- In the Netherlands Wageningen University, GD (Gezondeindsienst voor Dieren, Animal Health Service of the Netherlands)
- In New Zealand Massey University
- In Norway Norges veterinærhøgskole (Norwegian School of Veterinary Science)
- In the UK the Department of Agriculture and Rural Development of Northern Ireland (DARDNI), veterinary organisations in Northern Ireland (North of Ireland Veterinary Association, Association of Veterinary Surgeons Practicing in Northern Ireland), Defra (the UK Department of Environment, Food and Rural Affairs), Veterinary Laboratories Agency, Scottish Agricultural College, Royal Veterinary College

#### The TB Diagnostics and Immunology Research Centre

Staff from the Centre acknowledges the help and support of DVO staff throughout Ireland for their efforts and assistance in providing samples for the IFN- $\gamma$  test.

### The Badger Vaccine Project

Staff from the Badger Vaccine Project acknowledge Damien O'Meara, Marion Barret, Frances Quigley and staff at the mycobacteriology laboratory (Abbotstown, Ireland) for their contributions and assistance in the Badger Vaccine Project, and Paddy Sleeman of University College Cork for fieldcraft. Glyn Hewinson, Mark Chambers and staff at Veterinary Laboratories Agency (VLA, UK) are also thanked for developing and carrying out many of the immuno-assays used in the badger vaccine studies, and for contributing technical expertise and advice for the research program.

Some photographs kindly supplied by An Bord Bia.

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# **BOVINE TUBERCULOSIS**



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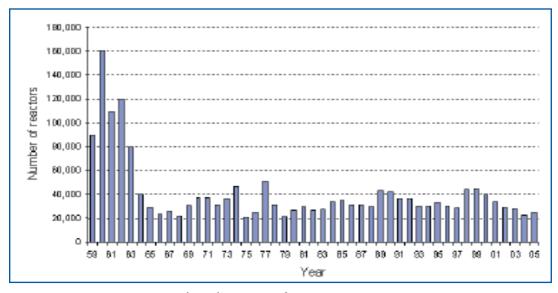
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### Overview

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

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



Tuberculin reactors from 1959 to 2005

### The Centre for Veterinary Epidemiology and Risk Analysis

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

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

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

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

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

### The TB Diagnostics and Immunology Research Centre

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

### The Badger Vaccine Project

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

### **Peer-Reviewed Papers**

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

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

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

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

• Badgers are an important contributor to the epidemiology of tuberculosis in Irish cattle 🤊

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

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

The results of the study have confirmed that badgers are an important contributor to the epidemiology of tuberculosis in Irish cattle. During the study period, there was a significant difference between the removal and reference areas in all four counties in both the probability of, and the time to, a confirmed herd restriction due to

tour (metres Buffer Area

Cork project area

5 Kilomete

tuberculosis. To illustrate, in the final year of the study, the odds of a herd restriction in the removal as compared to the reference area was 0.25 (95% CI 0.07-0.88) in Cork, 0.04 (0.00-0.27) in Donegal, 0.26 (0.08-0.79) in Kilkenny and 0.43 (0.22-0.84) in Monaghan. The validity of the study has been examined in considerable detail. Efforts to minimise badger-to-cattle transmission in Ireland must be undertaken in association with the current comprehensive control programme, which has effectively minimised opportunities for cattle-to-cattle transmission.

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

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

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

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

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

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

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

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

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

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

<sup>66</sup> There was little evidence of spatial clustering of tuberculous badger setts in each area <sup>99</sup> \*\* There is evidence of clustering of *M. bovis* strains at the macro-level (counties) in cattle and badgers, however, there was less evidence of clustering within counties and no clear spatial association between badger and cattle strains<sup>99</sup>

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

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

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

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

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

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

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

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

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

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

Wildlife reservoirs of *Mycobacterium bovis* are of importance due to the significant number of bovine tuberculosis breakdowns in cattle herds in both Ireland and in the United Kingdom. The badger (*Meles meles*) has been implicated, but it is unclear how the disease is transmitted to cattle from badgers. Few studies have addressed dissemination and persistence of environmental *M. bovis*. Survival of up to 6 weeks for *M. bovis* cells inoculated into soil and feces, detected by traditional selective cultivation methods has been reported. However, cultivation techniques for monitoring *M. bovis* and other mycobacteria in soil are impeded by the slow growth rates of *M. bovis* and the need for prolonged incubation of highly selective agars. In this study, we present the first report of the use of analysis of community DNA with specific PCR primers targeting both antigen genes and the *M. bovis* 16S rRNA gene to demonstrate the long-term survival of *M. bovis* in environmental samples. <sup>66</sup>This study provides clear evidence that *M. bovis* can persist in the farm environment outside of its hosts and that climatic factors influence survival rates<sup>99</sup>

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

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

n New Zealand the brushtail possum (*Trichosurus vulpecula*) is the principle wild life reservoir of bovine tuberculosis. BCG vaccine has been found to induce significant level of protective immunity. The number of routes by which BCG vaccine can be administered to free-ranging wild animals is limited. It was found that conjunctival vaccination with BCG induced a significant level of protective immunity against experimental *M. bovis* challenge. Conjunctival vaccination of possums with BCG induced significant protection equivalent to other routes of administration<sup>99</sup> The changed behaviour of possums in the terminal stages of tuberculosis increases the risk of transmission to cattle and deer<sup>99</sup> Ranging behaviour and duration of survival of wild brushtail possums (*Trichosurus vulpecula*) infected with *Mycobacterium bovis* 

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

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

A delay in processing of the blood samples may potentially result in *M. bovis* infected animals remaining undetected in a herd<sup>99</sup> The effect of the tuberculin test and the consequences of a delay in blood culture on the sensitivity of a gamma-interferon assay for the detection of *Mycobacterium bovis* infection in cattle

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

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

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

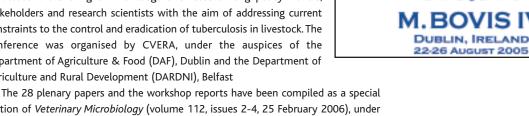
The epidemiology and pathogenesis of Johne's disease in cattle needs to be clarified Corner, L.A.L., Pfeiffer, D.U., Abbott, K.A., 2004. The respiratory route as a hypothetical aerosol transmission pathway for Mycobacterium paratuberculosis infection in cattle. Australian Veterinary Journal 82, 170-173.

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

### Published Reports, Conference Papers and Posters, Industry Papers

### 4th International Conference on Mycobacterium bovis

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



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

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

The conference was attended by over 300 scientists and veterinarians from 35 countries, highlighting the ongoing importance of bovine tuberculosis in many countries<sup>99</sup>







A critical review of progress in the national Irish control programme

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

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

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

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

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

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

### Other conference presentations and posters

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

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

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A report prepared for the Minister of Agriculture & Food December, 2004



### Work in progress

BOVINE TUBERCULOSIS

When the IFN-γ assay is used in parallel with the tuberculin test, it is capable of identifying infected cattle which might otherwise not be detected by the skin test until later, if at all<sup>99</sup>

#### **TB Diagnostics & Immunology Research Centre**

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

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

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

#### Development of a badger vaccine against tuberculosis

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

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

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

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

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

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

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

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

<sup>66</sup>The vaccine, when available, should serve the purpose of preventing disease in the badger, thereby breaking the chain of infection between badgers and cattle<sup>99</sup>

# Although cattle-tocattle transmission is now considered of lesser importance as a result of the national control programme, additional strategies may help to limit this further. Pre-movement testing is one such option<sup>99</sup>

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

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

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

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

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

Culling may influence the prevalence of tuberculosis in badger populations<sup>99</sup> The effect of varying levels of population control on the prevalence of tuberculosis in badgers in Ireland

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

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

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

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

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

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

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

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

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

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

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

An association was found between TB infected herds and proximity to badger setts. However, no association was found with proximity to infected badger setts Risk factors for tuberculosis in clear cattle herds that disclosed an animal with a tuberculosis lesion at slaughter during 2003 in Ireland

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

••Our findings support the widely accepted view that TB is a slowly progressive, chronicinfectious disease transmissible from cattle to cattle

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

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

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

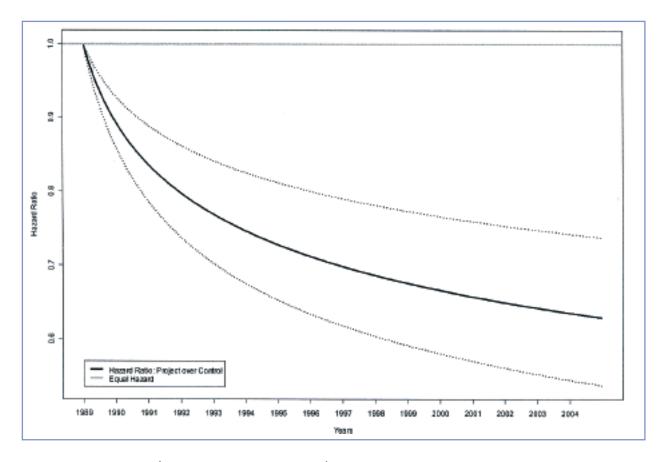
Our analysis showed that two factors seem to be of paramount importance; namely, the time that the index lesioned animal spent in the herd, and the presence (or otherwise) of a BTB-lesioned animal in a previous BTB episode. The risk posed by the presence of a lesioned animals during this episode is related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

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

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

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

The east Offaly and four area projects provide conclusive evidence of the link between the removal of badgers and the occurrence of bovine TB on Irish farms. It is now some years since the formal conclusion of the east Offaly project, and data are now available to assess the longer-term effects of proactive badger removal. Therefore, this study is seeking to assess the impact of badger removal on the disclosure of cattle tuberculosis, both during and following a formal period of proactive badger removal. A formal programme of badger removal was conducted in east Offaly from 1989-1995. This study is re-examining these data, but also subsequent data until the end of 2004. The data are analysed using survival analysis methods; specifically, a Cox regression model with the Anderson & Gill method for modelling multiple events. The model has project/control, herd size and previous history as time dependent covariates. Because the effect of being in the project area can change over time, it is being modelled using both polynomials and psplines. This study is investigating the long term impact of proactive badger removal on herd restrictions due to bovine tuberculosis<sup>99</sup>



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

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

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

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

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

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

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

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

By focusing on the minority of herds having extended restriction periods, this analysis will consider the possibilities for defining 'chronic herds'<sup>99</sup>

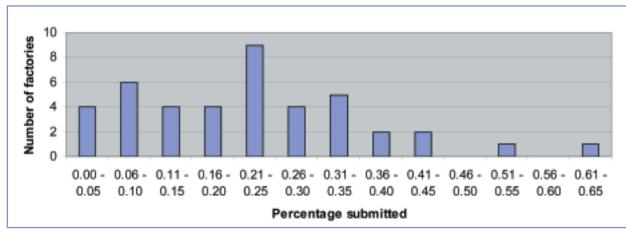
# Quantifying the relative efficiency of factory surveillance in the disclosure of tuberculous lesions in attested Irish cattle

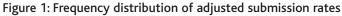
Principal investigators: Klaas Frankena, Ilsa van Grevenhof (Wageningen University), Paul White, James O'Keeffe and Simon More (CVERA)

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

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

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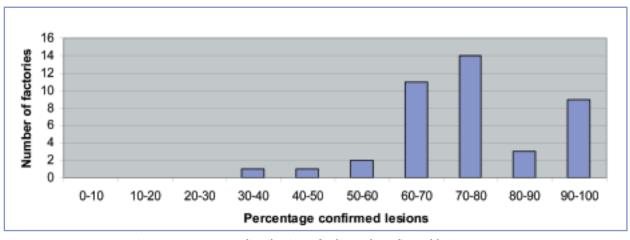


Figure 2: Frequency distribution of adjusted confirmed lesion rates

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

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

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

 A study is currently underway to determine the duration of protection in badgers from a single oral dose of BCG. The question is an important one. In the field we need to know how frequently revaccination will be required to provide adequate protection. Badgers were challenged at 3, 6 and 12 months after vaccination. Protection will be measured using immunological and pathological parameters.

- Several different immunodiagnostic assays for the diagnosis of TB in badgers have been developed. The sensitivity of these assays to detect infection in naturally infected animals has not been established. Studies are being carried out on blood from 215 badgers and compared to the isolation of *M. bovis*, the definition of infection.
- A study of the pathogenesis of infection in naturally infected badgers is being conducted. This study aims to determine the significance of the lungs as the primary site of *M. bovis* infection in naturally infected badgers. Samples from 215 culled badgers have been collected.

Knowledge of factors that influence an animal's response to the SICTT should improve our understanding of the current testing program in Ireland<sup>99</sup>

An outbreak of TB in

alpaca is currently being

Investigated 99

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

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

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

### Bovine tuberculosis in alpaca

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

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

A series of studies is being undertaken in support of BCG vaccination of badgers as a control tool in the national programme<sup>99</sup>

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### An understanding of the spatial distribution of TB prevalence in badgers

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

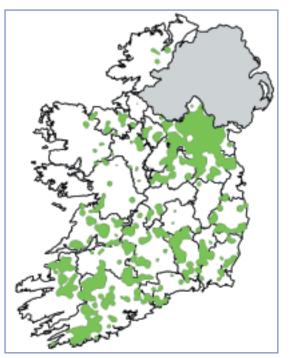
The control of the transmission of *Mycobacterium bovis* from badgers to cattle is currently being achieved through focused culling, following a detailed veterinary epidemiological investigation. As part of the national programme, work is currently underway towards the use of BCG vaccination to control TB infection in badgers. This process would be greatly aided if the geographic distribution of infection in the badger population were understood. This study specifically seeks to determine whether there is spatial variation in the prevalence of tuberculosis in badgers. <sup>66</sup>We require an understanding of the spatial variation in TB prevalence in badgers as we plan for the use of BCG vaccine in badgers<sup>99</sup>

### Work of the Wildlife Unit

### Principal investigator: James O'Keeffe (CVERA)

A compulsory national bovine tuberculosis eradication program has been operating in the Republic of Ireland since 1959. Substantial progress was achieved in the early decades, but since the mid 1970s there has been no improvement despite the continuing application of a very intensive national tuberculin testing program. Geographical information systems techniques including kernelling have been used to identify areas of the county where tuberculosis is consistently identified at high incidence levels. Each year, circa 70% of all standard "skin test" reactors are drawn from roughly 30% of the area of agricultural land. Local densities of badgers are being reduced, with removal operations being more intense in areas of the country where TB has been particularly problematic. In the short term, this will result in lowering the risk of cattle herds becoming infected with TB from TB infected badgers in the local

environment. The frequency of significant cattle/badger interactions will be reduced as a consequence of the local reduction in the density of both cattle and badgers. Badger interventions are carefully planned and rigorously monitored and only take place as a sequel to an epidemiological investigation carried out by State Veterinarians who must follow a standardised protocol. The medium term strategy targets a 25-30% reduction in the national badger population. This strategy will be re-visited when the results of planned vaccine trials have been evaluated. <sup>66</sup>Reactive badger removal forms part of the short term stratergy to control bovine tuberculosis in Ireland<sup>99</sup>



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

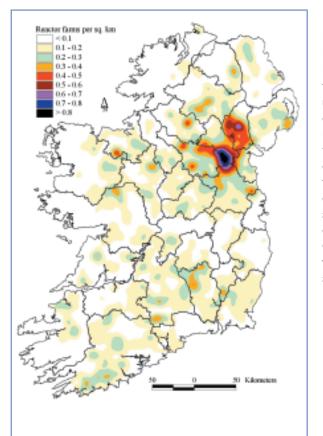
### New work

#### New measures of progress

Principal investigators: Simon More and Isabella Higgins (CVERA)

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

### North-South collaborative mapping



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

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

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

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

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

Reactive badger removal forms part of the short-term strategy to control bovine budger removal on subsequent bovine tuberculosis in cattle herds in Co. Laois.

### **Tuberculin registration**

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

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

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

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

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

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

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

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

### Study of the source of infection in cattle breakdowns

Principal investigators: Tracy Clegg and Simon More (CVERA)

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

### Investigating an outbreak of TB with human involvement

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

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

### Badger distribution and abundance: results from the four area project

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

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

### The impact of badger removal in the four area project

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

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

### Winter yard survey

#### Principal investigator: Paddy Sleeman (University College Cork)

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

### Genetics of predisposition to tuberculosis in Irish dairy and beef cattle

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

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

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

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

- The first chapter of the PhD has been considered previously (Descriptive statistics, 1995-2004). The objectives of the subsequent chapters include:
- to quantify the contribution of introduced, possibly infected animals as source of herd breakdown (Chapter 2);
- to incorporate the concept of the reproduction ratio (R<sub>o</sub>) into measurement of the between-herd and within-herd spread of tuberculosis (Chapter 3);
- to estimate the component badgers contribute to the observed levels of tuberculosis evident in cattle herds (Chapter 4); and
- based on the results of previous chapters, to adopt a modelling approach to combine the risk factors from various sources, and calculate an overall R<sub>o</sub> value (Chapter 5)

### National badger census

Principal investigator: Guy McGrath (CVERA)

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

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

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

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

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

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

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

### The effect of bovine TB on milk production

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

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

### Spatial analysis of bovine TB data - the effect of badger removal

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

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

### The Greenfield study

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

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

### Key meetings/presentations

#### Tracy Clegg

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

### Leigh Corner

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

#### Eamonn Gormley

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

### Gabrielle Kelly

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

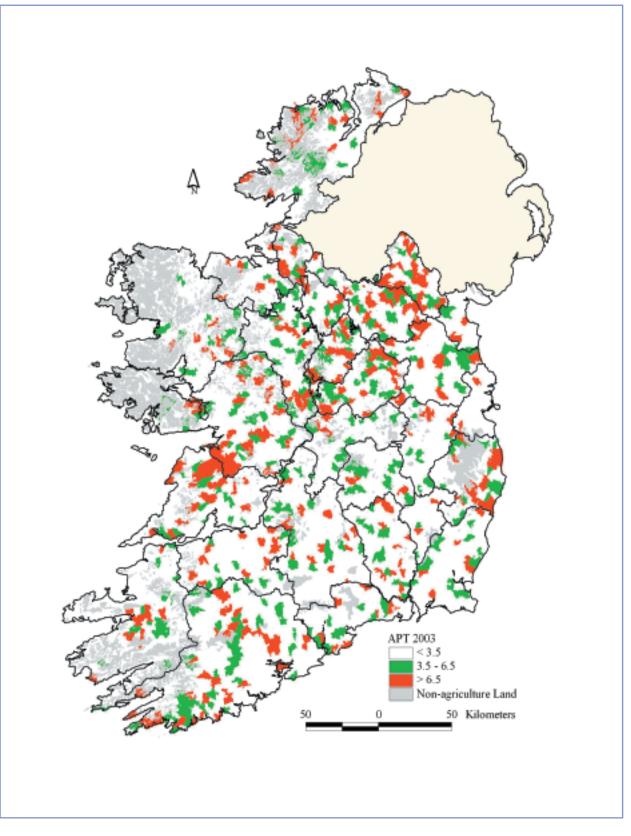
### Simon More

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

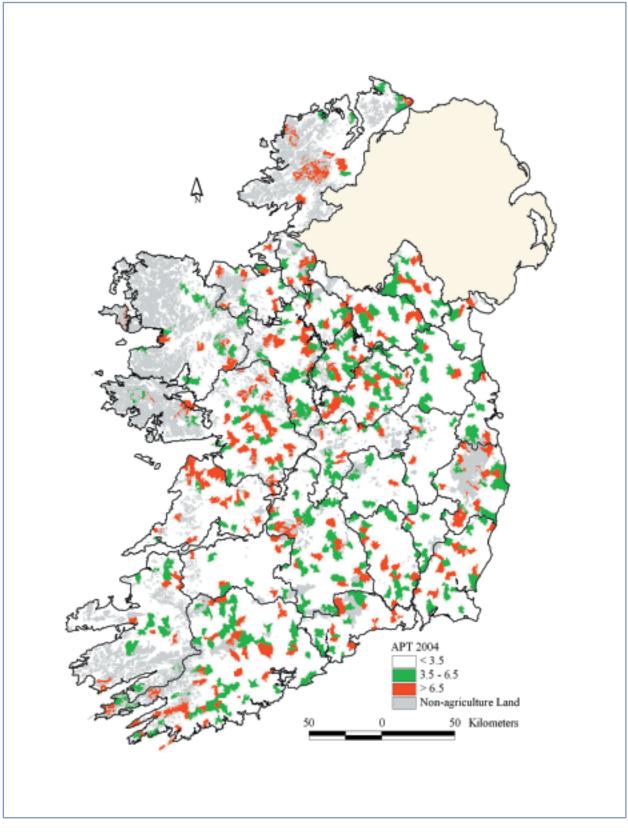
### Paul White

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

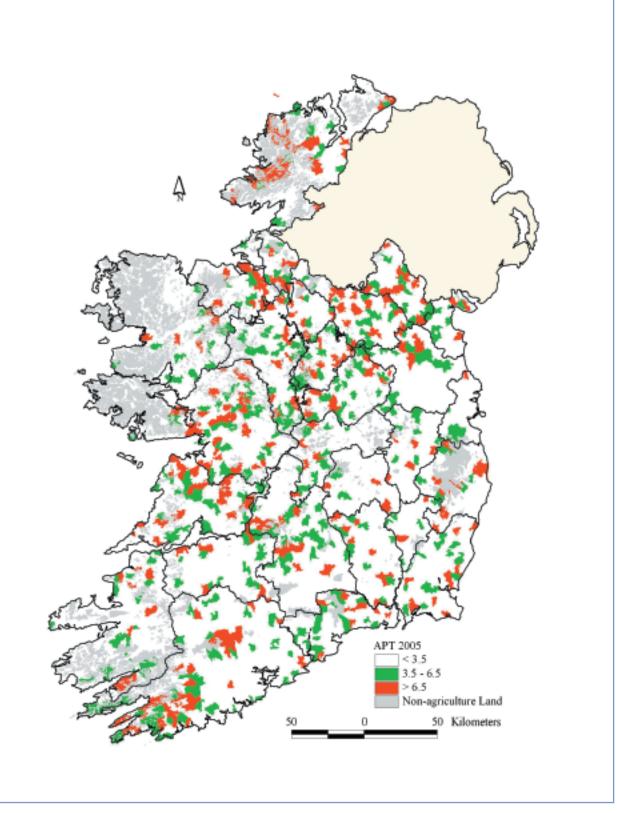
## National maps



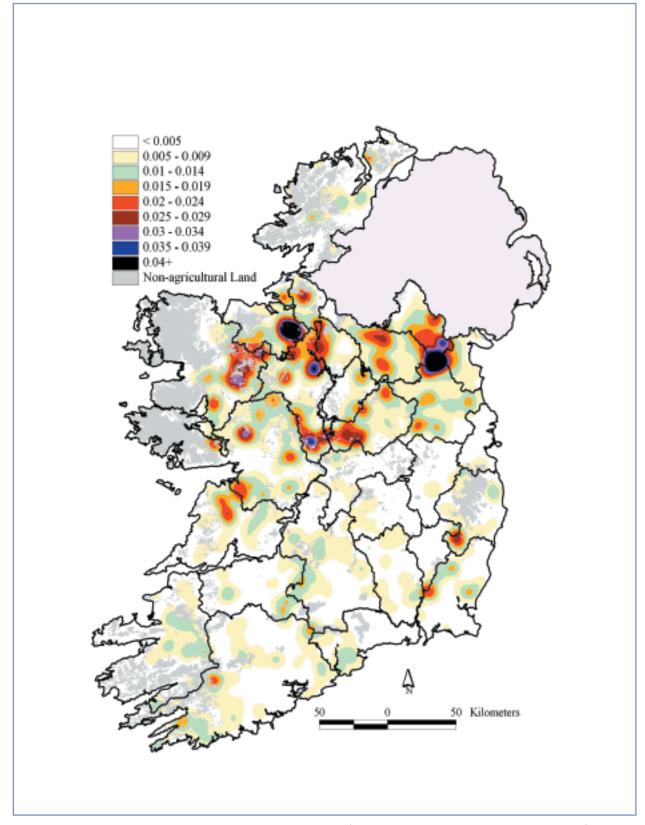
APT (reactors per 1000 tests) per district electoral division, 2003



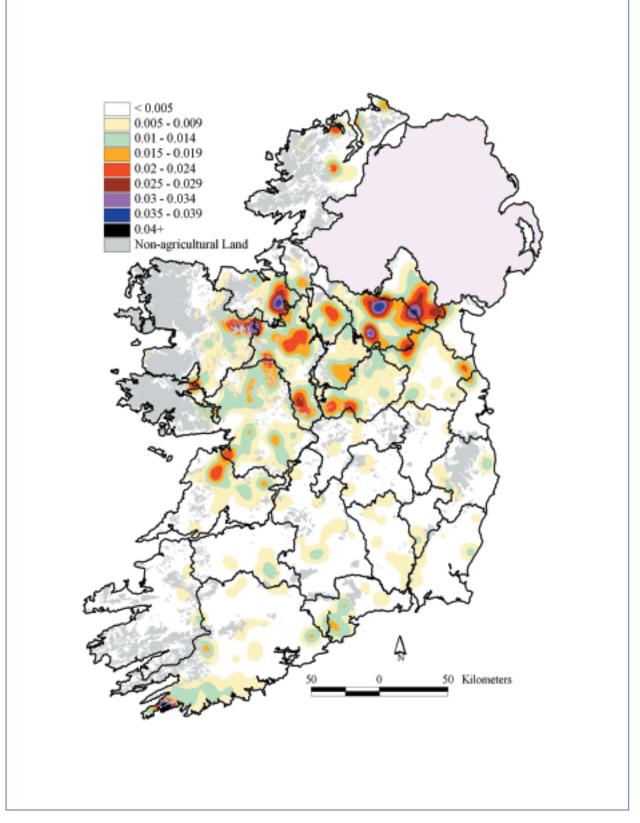
APT (reactors per 1000 tests) per district electoral division, 2004



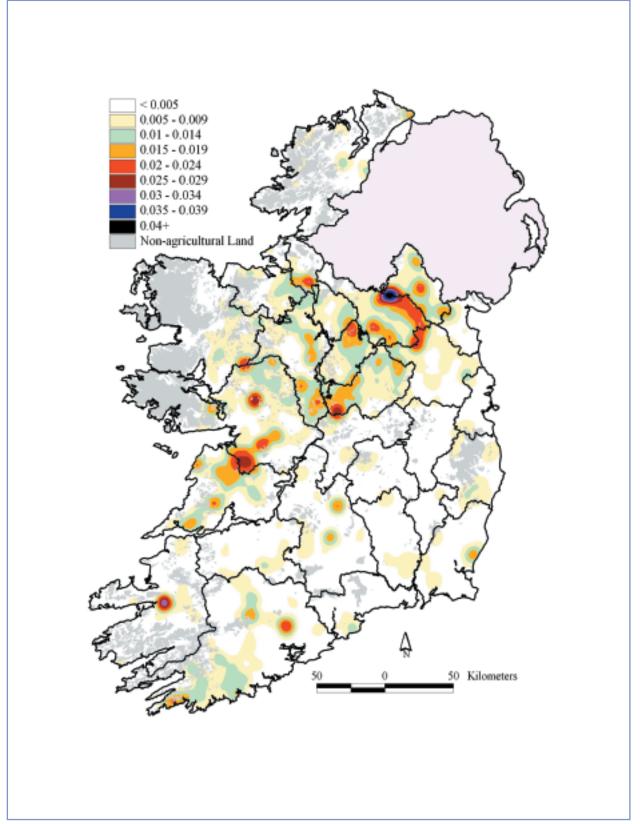
APT (reactors per 1000 tests) per district electoral division, 2005



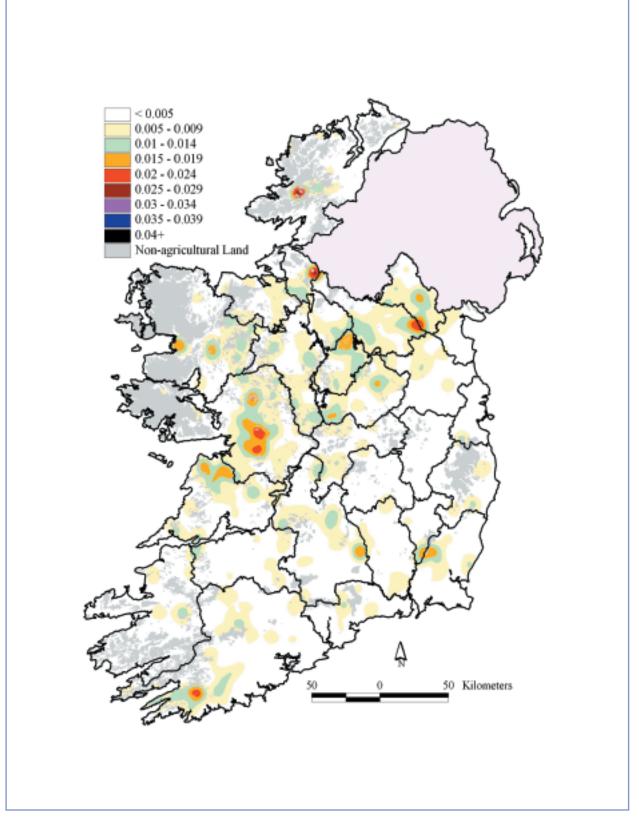
Density of TB incidence per square km during 2001 (kernel density with search radius at 10km)



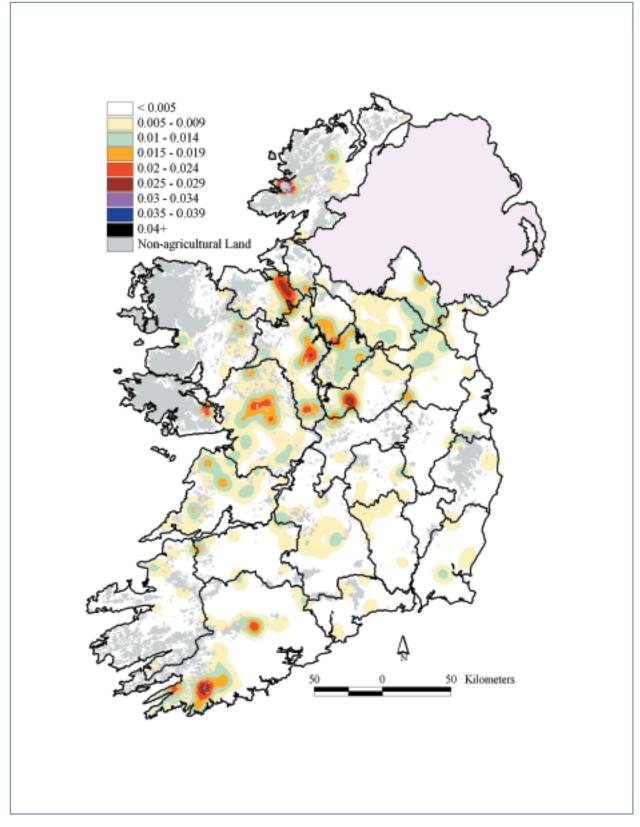
Density of TB incidence per square km during 2002 (kernel density with search radius at 10km)



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



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



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



# BOVINE BRUCELLOSIS



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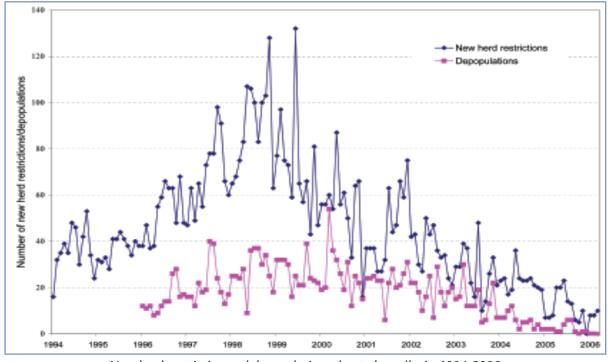
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### Overview

A national programme to eradicate bovine brucellosis from Ireland commenced in 1965. At this time, between 12% and 15% of herds were infected, with disease incidence being higher in the south than the west and north-west. Early progress towards eradication was good, with herd prevalence falling to approximately 0.2% by 1986. By this date, herd restrictions were limited to north Cork, Limerick and Tipperary. However, these rapid gains were not sustained and brucellosis re-emerged as a significant problem in Limerick and Tipperary in the late 1980s. By 1992 the number of herd restrictions had increased in Limerick and Tipperary, and disease was also detected in counties that had been clear for a number of years. A number of additional controls were introduced nationally in the late 1990s. The herd prevalence subsequently peaked at 0.74% in 1998 (Griffin and Collins 1999), and has since been in steady decline. Of 126,084 herds nationally, 323 (0.3%) herds were restricted and 68 of these (0.05% of all herds) were depopulated during 2004.

As part of the Irish brucellosis eradication programme, annual serological testing is conducted on all female cattle and bulls over one year of age. Additional serological testing is also carried out on herds that are contiguous to disease, on animals pre- and post-movement, as well as on animals that have been traced from diseased premises. The whey ELISA test are carried out on bulk milk tank samples and all abortions should be notified to the Dept. of Agriculture. Cull cows are also tested at the point of slaughter. Herds that test positive for brucellosis have movement restrictions imposed and if disease is thought to be present will be depopulated.

Work within CVERA has sought to contribute information to assist with national decision-making. In collaboration with colleagues in the field, detailed investigations have been conducted on several recent outbreaks of brucellosis to determine the source of infection, likely mechanisms for spread and other farms at-risk. Studies have also been conducted to determine the dispersal and survival of a defined cohort of Irish cattle, and to undertake a descriptive analysis of data routinely collected at brucellosis herd de-restriction during 2000. Finally, as Ireland moves towards effective eradication, the interpretation of diagnostic tests may become increasingly problematic. Work is currently underway to critically evaluate the true disease status of Irish cattle herds with inconclusive evidence of bovine brucellosis.



New herd restriction and depopulations due to brucellosis, 1994-2006

## Work in progress

<sup>66</sup>Outbreak investigations have been conducted to gain a detailed understanding of the source and spread of infection<sup>99</sup>

### Investigating outbreaks of brucellosis

Principal investigators: Martin Hayes (CVERA), Ascinta Kilroy (DAF), Simon More and Seán Ashe (CVERA)

With the ongoing drop in disease incidence, outbreaks are becoming less common and (often) more isolated. These outbreaks present new challenges for those overseeing the national disease eradication programme. The purpose of these investigations is to gain a detailed understanding of the source and spread of infection. It is hoped that this information will contribute to ongoing improvement in national and local disease control efforts. In these investigations, DAF staff, relevant farmer(s) and CVERA staff worked in partnership to examine relevant epidemiological and other information.

<sup>66</sup>Data collected by veterinary inspectors at the time of herd de-restriction are being used to gain insights into the epidemiology of bovine brucellosis in Ireland<sup>99</sup> Descriptive analysis of data routinely collected at brucellosis herd de-restriction during 2000

Principal investigators: Seán Ashe (DAF), Martin Hayes, James O'Keeffe and Simon More (CVERA)

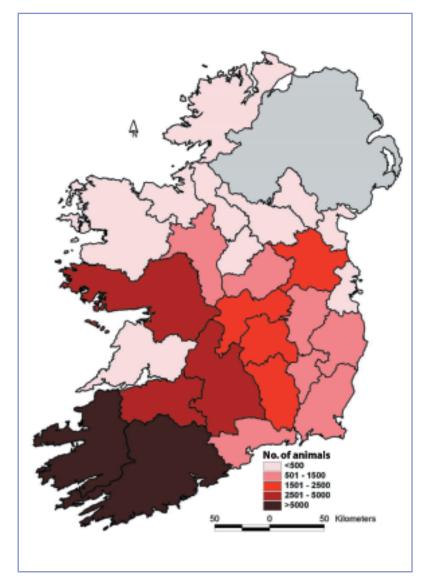
In the year 2000, field veterinarians gathered information from herds at the time of de-restriction from an earlier brucellosis outbreak. These data were used to gain an insight into the epidemiology of brucellosis on these farms and the local management of the eradication programme. Key data were collected to enable the spatial and temporal distribution of cases to be described, as well as methods of disease identification, the likely source of disease, characteristics of isolation and culture technique, and the overall disease risk associated with restricted herds that were and were not subsequently depopulated. This database is currently being analysed.

### Dispersal and survival of a defined cohort of Irish cattle

Principal investigators: Seán Ashe (DAF), Simon More, James O'Keeffe and Paul White (CVERA)

A nunderstanding of livestock movements is critical to effective disease prevention, control and prediction. As yet, livestock movement in the Republic of Ireland has not been quantified. This study has sought to define the survival and dispersal of a defined cohort of cattle, born in county Kerry during 2000. The cohort was observed for a maximum of four years, from 01 January 2000 to 31 December 2004. Beef and dairy animals moved an average 1.31 and 0.83 times, respectively. At study end, 18.0% of the beef animals remained alive on Irish farms, including 6.7% at the farm-of-birth, compared with 48.5% 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 4-year survival probability was 0.09 (male beef animals), 0.27 (male dairy), 0.39 (female beef), and 0.77 (female dairy). Although there was considerable dispersal, the number of moves per animal was less than previously thought.

An understanding of livestock movements is critical to effective disease prevention, control and prediction<sup>99</sup>



The location, on 1 January 2002, of beef cattle born in County Kerry during 2000

As Ireland moves

towards effective

eradication, the

interpretation of

diagnostic tests may

become increasingly

problematic<sup>99</sup>

A critical evaluation of the true disease status of Irish cattle herds with inconclusive evidence of bovine brucellosis

Principal investigators: Martin Hayes, Seán Ashe (DAF), Daniel Collins, Simon More (CVERA), Seamus Power, Kevin Kenny, Michael Sheahan and Garry O'Hagan (DAF)

As Ireland moves towards effective eradication, the interpretation of diagnostic tests may become increasingly problematic. With this in mind, work is currently underway to critically evaluate the true disease status of Irish cattle herds with inconclusive evidence of bovine brucellosis. All cattle herds in Ireland are subjected to an annual round test, with blood being collected from all breeding cattle of 12 months of age or greater. In this study, results from all testing conducted in Ireland during 1 September 2004 to 31 August 2005 were used to identify and classify all herds with any evidence of a serological response to testing, as follows:

- Group A herds, where at least two animals had a CFT result of 111 international units or more at the annual test, and/or clinical evidence of abortion in association with serological evidence of infection with Brucella abortus, and or bacteriological evidence of B. abortus from any herd sample;
- *Group B herds*, where one or more animals had a positive CFT result, but the Group A herd criteria were not met; and
- *Group C herds*, where one or more animals had a positive ELISA result, but the Group A and/or B herd criteria were not met.

Detailed examination of these herds following initial detection was constrained by the nationally-accepted disease control policy. For example, Group A herds are depopulated as a matter of urgency. When feasible, detailed epidemiological and further testing data were collected following the initial positive testing result. Each of the study animals was re-bled for testing using MSAT, CFT and ELISA. Further, a brucellin skin test was conducted by the field veterinary inspector on the CFT-positive study animals, coinciding with the re-bleed visit. The status of a range of risk factors and related data was also collected. When CFT-positive animals were removed, retropharyngeal and mammary lymph nodes were collected for culture at the State Veterinary Laboratory at Abbotstown. A post calving serological test will also be carried out on the remaining animals of the study herds to determine the future disease status of study herds. Analysis of these data is ongoing.

New work

### Whole-of-island tuberculosis and brucellosis statistics

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

The whole of the island of Ireland can be considered a single epidemiological unit, particularly with the pending removal of restrictions to the movement of cattle from north to south. Consequently, effective disease control will increasingly rely on shared information for improved decision-making, particularly in border counties. At a recent N-S meeting on 11 October 2005, it was agreed that whole-of-island tuberculosis and brucellosis statistics should be created. This project will seek to act on this decision, in collaboration with northern colleagues.

## Key meetings/presentations

Simon More, Martin Hayes, Seán Ashe

- DAF meeting (ER19B) (Longford, 24 May 2005; Kilkenny, 25 May 2005)
- DAF meeting (brucellosis) (Drumshanbo, 7 October 2005)

BOVINE BRUCELLOSIS



TSE

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### Overview

The first case of Bovine Spongiform Encephalopathy (BSE) confirmed worldwide occurred in an animal which died in the UK in 1985 (Wells et al., 1987). The first confirmation of BSE in Ireland occurred in 1989 (Sheridan et al., unpublished). In response to the identification of BSE in Ireland, the Department of Agriculture and Food (DAF) introduced a series of control measures which relied, as the UK measures did, on a ban on the direct feeding of meat and bone meal (MBM) to ruminant animals.

Cases of BSE continued to be confirmed sporadically in Ireland between 1989 and 1995 (average 15 cases annually). The epidemiological features of the cases confirmed in Ireland did not differ in any significant way from those described by Wilesmith et al in 1988 (Griffin et al., 1997). By the end of 1996 it was clear both in Ireland and in the UK that the MBM ban, though capable of controlling disease, was not capable of completely preventing transmission. Several authors (Denny and Houston, 1997; Hornlimann et al., 1997; Stevenson et al., 2000) suggested that the cause of these so called BAB (born after the ban) cases was inadvertent cross contamination of ruminant rations with MBM intended for use in pig and poultry rations. In response to these findings, and the critical repercussions of the UK announcement in March 1996 that 10 cases of a novel disease (now called variant Creutzfeldt-Jakob disease) linked to BSE had been confirmed in human patients, the Department of Agriculture comprehensively reviewed its BSE control strategy, replacing it in 1996 and 1997 with a cumulative risk management strategy aimed at eradication.

Following the introduction of these controls, CVERA began work on a model based on that first used by Anderson et al, in 1996. The purpose of this model was to predict the number of cases per year, assuming that feed borne transmission had been eliminated. Comparison of predicted values with observed values enabled the effect of the enhanced control measures to be measured. Accuracy of this model has been assisted by the results of the active surveillance programme introduced throughout the European Union in 2001 which increased the ability of Member States to identify cases.

Although the controls introduced in Ireland in 1996 and 1997 have been very effective, BSE has been confirmed in a small number of animals born after their introduction (16 cases). Work is currently underway at CVERA with a view to identifying the route through which these animals were exposed.

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Hornlimann, B., Audigé, L., Somaini, B., Guidon, D., Griot, C., 1997. Case study of BSE in animals born after the feed ban (BAB) in Switzerland. Épidémiologie et Santé Animale 31/32: 11.08.1-11.08.3.

Stevenson, M. A., Wilesmith, J. W., Ryan, J. B. M., Morris, R. S., Lockhart, J. W., Lin, D., Jackson, R., 2000. Temporal aspects of the epidemic of bovine spongiform encephalopathy in Great Britain: individual animal-associated risk factors for the disease. Veterinary Record 147: 349-354.

Wells, G. A. H., Scott, A. C., Johnson, C. T., Gunning, R. F., Hancock, R. D., Jeffrey, M., Dawson, M., Bradley, R., 1987. A novel progressive spongiform encephalopathy in cattle. Veterinary Record 121: 419-420.

Wilesmith, J.W., 1998. Manual on bovine spongiform encephalopathy, Food and Agricultural Organization, Rome.

### Peer-reviewed papers

These papers have been written in collaboration with colleagues from Canada, Ireland and Sweden.

The model was used to assess the nature of the disease propagation, to predict the number of future cases and to assess the risk to humans in terms of the number of infected animals that were processed in Ireland<sup>99</sup>

### Analysis and prediction of the BSE incidence in Ireland

Pawitan, Y., Griffin, J.M. and Collins, J.D., 2004. Analysis and prediction of the BSE incidence in Ireland. Preventive Veterinary Medicine, 62: 267-283.

Donnelly and Ferguson (2000) have described detailed epidemiologic models for DBSE from the classical mathematical modelling route. The purpose of this paper is to report on the statistical methods that were used to model Irish BSE data. The model was a simplified version of the British models. However, it contained some novel aspects including the development of a numerical method to describe the model parameters, inclusion of over-dispersion in the model and the development of appropriate bootstrap procedures for estimation and prediction. It was fitted to the available data as a nonlinear Poisson regression model. The model was used to assess the nature of the disease propagation, to predict the number of future cases and to assess the risk to humans in terms of the number of infected animals that were processed in Ireland.

<sup>66</sup>Among BSE case herds identified in Ireland between 1996 and 2000, one statistically significant primary spatial cluster and one statistically significant secondary spatial-temporal cluster both of which may be associated with feed source can be detected<sup>99</sup> A temporal-spatial analysis of bovine spongiform encephalopathy in Irish cattle herds, from 1996 to 2000

Sheridan, H.A., McGrath, G., White, P., Fallon, R., Shoukri, M.M. and Martin, S.W., 2005. A temporal-spatial analysis of bovine spongiform encephalopathy in Irish cattle herds, from 1996 to 2000. Canadian Journal of Veterinary Research, 69: 19-25.

his study describes a spatial-temporal analysis of BSE case herds identified in Ireland in the years 1996 to 2000. Geographical clustering suggested by spot and Standardised Morbidity Ratio (SMR) maps was investigated using SatScan Version 2.1. A statistically significant cluster (P=0.0001) without a temporal element was detected in the northeast of the country centred on County Monaghan. A second statistically significant cluster (P=0.001) with a temporal component (1992-1994) was detected in the southeast centred on County Wexford. Both clusters remained significant after correction for confounding by herd size and enterprise type though this correction did remove the statistical significance of a third cluster detected in the south centred on County Cork. Although inclusion of the co-ordinates of major feed suppliers increased the power of the analysis, models were not able to agree on the feed supplier at the centre of either the primary or secondary cluster. The study provides evidence that BSE herds in Ireland cluster geographically. The factor/s responsible for clustering are more likely to be associated with the herd where the case animal was located at the time of infection (putative exposure herds); thought to occur under one year of age. The study provides evidence of a spatial association between feed supplier and clusters of putative exposure herds.

## Work in progress

### BSE cases in Ireland in post-1997 born animals

Principal investigators: Hazel Sheridan, Rob Doyle (DAF) and Simon More (CVERA)

To date, 16 cases of BSE have been confirmed in animals born after 1997. Of these 16, 4 animals were born in 1998, 7 in 1999, 3 in 2000 and 2 cases were born in 2001.

As described by Dr Wilesmith in the UK, such cases fit the pattern of the 'third' epidemiologically distinct series of cases of which more than 100 have been diagnosed in the UK to date. The first series occurred prior to the introduction of a ban on meat and bone meal (MBM) when cattle were fed contaminated MBM directly. The second occurred between the initial ban and the re-enforced ban and have been attributed to the cross contamination of ruminant rations with MBM intended for use in pig and poultry ration. The third series have occurred in animals born after the enhanced controls (Ireland 1996/1997; UK 1996). The cause of such remains unproven and is still the subject of debate.

In all cases of BSE confirmed in Ireland in animals born after 1997, a detailed investigation has been or is being carried out focussing on the farm in which the case animal spent its first year of life in an effort to establish:

- · What feeds which the positive animal may have received in its first year of life;
- Any other feeds which may have been present on the farm when the animal was in it's first year of life;
- Any material which may have been spread on land or used in the environment of the farm on which the animal spent its first year of life has been inspected;
- · Any medicines or remedies which were administered to the animal;
- The fate of the animal's dam.

The investigation into 12 of the 16 post-1997 born cases has been completed. Beyond the fact that all cases received concentrate feeding in their first year of life, no factor common to all farms has been found. Both suckler and dairy enterprises have been affected. Cases have occurred throughout the country both in counties with a relatively high incidence in previous years but also in counties which had a low incidence in years gone past. 6 of the cases have occurred on farms, which had another case of BSE either in the same cohort (2 farms have produced 2 post-1997 cases each) or in previous years (2 cases). The dam in 11 of the 12 completed investigations was dead by the time the case was detected. In the one case where the dam was still alive, the dam was purchased and held on the Department's research farm for 2 years. No signs were observed in the animal before slaughter and the animal produced a negative result to a rapid test at the time of slaughter. In 11 of 12 completed investigations, there was no evidence that blood, MBM, factory or knackery waste had been spread on the holding. In one case, adult bovine carcasses had been dumped on land adjacent to where the positive animal spent its first grazing season. Evidence of scavenger activity both at the dump and on surrounding land was detected. A number of farms had working dogs on the holding or an adjacent holding though no evidence could be found (with the exception of the case listed above) that these dogs had been fed on fallen animals. No evidence was found on any holding of failure to comply with the very stringent controls introduced in Ireland in 1996 to prevent unauthorised access to MBM or accidental contamination of ruminant feed with MBM intended for use in pig and poultry rations (with the possible exception of dry dog food). On 4 of 9 affected holdings, evidence of trace amounts of terrestrial land animal bone (sometimes with or without fish bone) were found in feed storage areas and/or disused silos. No evidence could be found that this material had been recently introduced; instead evidence existed that this material had been present on the farm for many years.

The route through which the small number of animals confirmed positive for BSE (16) born after 1997 (one year after the introduction of enhanced feed controls) were exposed remains uncertain though there is no evidence that the cases are linked to noncompliance with existing feed controls<sup>99</sup>

Given the number of cases, it is considered unlikely that these cases reflect spontaneous occurrence unless spontaneous disease in cattle is much more common than sporadic CJD in humans. Likewise, maternal transmission is considered unlikely and no evidence could be found of horizontal, iatrogenic or environmental transmission with the exception of the case described in the previous paragraph. Given this and what is known about the transmission of disease in the past, feed borne exposure remains the most likely source of infection in these cases. The precise mechanism by which this has occurred remains unknown. Several theoretical possibilities exist which have yet to be tested in any statistical sense. For example, EU wide feed controls to prevent cross contamination of ruminant rations with MBM intended for other uses did not come into force until January 2001. Given that mainland Europe and Ireland import a considerable amount of feed and feed ingredients, it remains a possibility that the 'infectious' feed could have been introduced from abroad. Also given the findings with regard to terrestrial land animal bone, it remains a possibility that cases could have had access to trace amounts of pre-1996 feed material lying around farms. It should be noted that the amounts were very small and they had been there a considerable length of time. Given that some farms had used dry dog food, which may have contained MBM, it remains a possibility that cases were caused by accidental incorporation of dry dog food in rations intended for ruminant use. Likewise, a theoretical possibility exists that animals were exposed to disease through the spreading of slurry from pigs which had been fed with feed containing MBM (allowed in Ireland until the EU ban in 2001). However, given the regulations regarding the removal of SRMs and pressure treatment of MBM before placing on the market, it is considered unlikely even if cattle had access to such material that it would have been capable of causing disease.

In summary, the cause of these post 1997 cases remains uncertain. There is no evidence that they were caused by a failure to comply with domestic provisions to enhance the feed ban. Cases will continue to be investigated as they emerge though it seems likely at this stage that the cause of these cases may never be known. Disappearance of these cases over time may support a hypothesis that they were caused in some way by residual feed risk, whether this be from feed manufactured before EU wide feed controls or residues on farm. This risk should decline over time.

Year of Birth	1989	1990	1991	1992	1993	1994	1995	1996	1997	1998	1999	2000	2001	2002	2003	2004	2005	Total
1981	1																	1
1982	2																	2
1983	1	1																2
1984	6	2	3		1													12
1985	4	8	5	1	2								1					21
1986	1	3	8	8	3	3		2					1	2	1	1		33
1987			1	7	4	3	2	1	2	1				2				23
1988				2	5	7	3	3		2	1	2	1	4				30
1989					1	3	5	8	2	2		1	1	1	4	3		31
1990						3	5	20	11	2	2	1	1	9	2	1		57
1991							1	24	24	8	3	3	7	10	6	3	1	90
1992								16	28	25	13	8	8	10	8	13	2	131
1993									12	29	40	30	19	42	23	16	13	224
1994									1	14	30	44	52	55	34	20	22	272
1995											6	54	115	130	70	39	16	430
1996												6	40	61	32	21	7	167
1997														5	2	3		10
1998																4		4
1999														2		2	3	7
2000																	3	3
2001																	2	2
Total	15	14	17	18	16	19	16	74	80	83	95	149	246	333	182	126	69	1552

Number of BSE cases in Ireland by year of birth and year confirmed, to end of 2005

### Modelling the demographics of a cattle population based on data from Ireland

Principal investigators: Jarlath O'Connor (DAF), Eamonn O'Leary (UCD), John Griffin (DAF) and Simon More (CVERA)

There is a paucity of information on the demography of cattle populations even though many countries have large databases of individual animal data. Some databases are more complete than others with regard to birth and exit data. The Department of Agriculture and Food (DAF), in Ireland, has a database containing all births (1996 onwards) and exits (2000 onwards) in the cattle population. The data has for the most part not been assessed from a demographic perspective. These data were initially recorded to ensure traceability of beef in light of BSE concerns.

The aim of the project is to develop and validate a demographics model for cattle based on data from the DAF database.

The demographics model is based on cohort life tables as used in human actuarial studies. Demographic processes such as births and deaths affect the size and composition of a population. Cohort life tables are tables of data on survivorship within a population. The standard method used to construct life tables is to collect data on cohorts, or groups of individuals all born in the same time period. These are used to determine mortality rates and survivorship, which in turn can be compared from cohort to cohort allowing analysis of their annual variation.

Important future applications of modelled data would be to give a temporally precise count of the cattle population, to look at trends in births and deaths stratified by age, sex or breed, to simulate the effects of ecological processes such as disease on the cattle population, and to help policy makers in the decision making process.

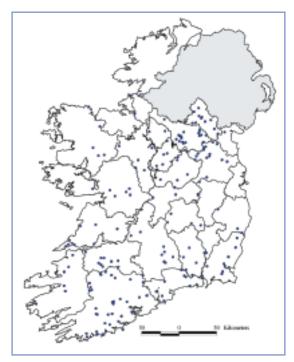
Work is underway to model the demographics of the Irish cattle population<sup>99</sup>

## Key meetings/presentations

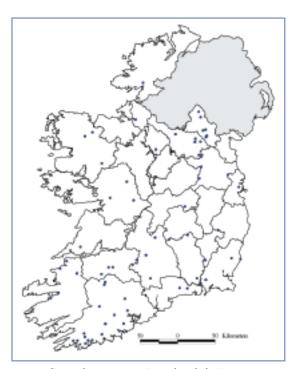
## Hazel Sheridan and Simon More

- DAF meeting (BSE investigations; Sligo, 9 February 2005)
- DAF meeting (BSE investigations; Sligo, 1 June 2005)

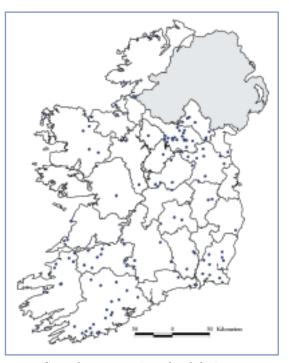
## National maps



Confirmed BSE cases in Ireland during 2003



Confirmed BSE cases in Ireland during 2005



Confirmed BSE cases in Ireland during 2004



# HERD HEALTH



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### Overview

Livestock farming in Ireland is at the start of a period of substantial challenge. The value of Irish product has come under considerable pressure, as a result of increasing international competition. There are also clear signs of reduced subsidy support for Irish farmers (currently an average of 35% of annual farm income), as a result of decisions both from within and outside the European Union (EU). As agreed at the 6<sup>th</sup> Ministerial Conference of the World Trade Organization in Hong Kong, export subsidies will be phased out by 2013. Further, at the recent EU summit in December 2005, it was agreed that the European Commission will hold a 'full and wide-ranging' review of all EU spending, which includes the Common Agricultural Policy.

In a global market, competitiveness is closely linked with cost and value. In contrast to many key competitors, the cost of inputs on Irish farms is very high, as illustrated by the high level of wages and cost-of-living. Therefore Ireland cannot hope to compete on price. Logically, product quality will become increasingly important to the survival of Irish agriculture. This will require an increased emphasis on all aspects of quality, including animal health and welfare. An emphasis on animal health and productivity will be important both nationally (to facilitate market access and competitive advantage) and on-farm (positively influencing on-farm profitability by reducing cost per unit output and enabling production of high-quality product).

In response to these challenges, CVERA is playing a central role in the development of a national herd health initiative. Associated pilots, and the herd health reports, are important components of this broader initiative.

## Published reports, conference papers and posters, industry papers

#### The herd health initiative

More, S.J. and Barrett, D., 2005. The herd health initiative. Irish Veterinary Journal, 58: 692-694.

n a global trading environment, product quality will become central to the long-term survival of Irish agriculture. The herd health initiative, a partnership between government and a range of industry bodies and service-providers, is currently under consideration, to provide national leadership in the area of non-regulatory herd health and productivity. The initiative adapts international models of success (notably, from the Netherlands, Sweden and Australia) to the Irish context, and will seek to establish the national infrastructure that is needed to enable Ireland to achieve international best-practice in these areas. Based on International experience, industry leadership will be critical.

## Work in progress

To remain competitive, Ireland must achieve international bestpractice in the areas of herd health and productivity<sup>99</sup>

#### The herd health initiative

As highlighted previously, Irish agriculture is facing some difficult challenges and must produce at high levels of efficiency if it is to remain competitive. There are many production diseases at farm level that may not produce obvious clinical disease *per se* but do have significant impact on production efficiency. Addressing these issues presents both challenges and opportunity.

In recent months, a number of organisations have been working on the concept of an industry-led 'Herd Health Initiative' (HHI). These organisations have each clearly identified the need for Ireland to achieve international best-practice in the areas of herd health and productivity. Working with these bodies, CVERA has developed a proposal for a formal HHI that will drive non-regulatory issues (that is, those outside the direct remit of government) including:

- Those with a strong biosecure component, such as Johne's disease, IBR and BVD; and
- Those that generally do not have a strong biosecure component, including mastitis (and milk quality more generally), fertility and lameness.

The proposal envisages a central, not-for-profit, body, guided by key stakeholders (from industry, producers, government and relevant service providers) being created to provide central leadership in efforts towards continuous improvement in herd health and productivity.

Detailed problemsolving is a first step to solving on-farm milk quality problems

### The mastitis pilot

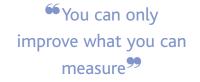
This pilot project has sought to demonstrate that veterinary interventions can positively contribute to milk quality, particularly among herds with long-term somatic cell count problems. Three regions, namely the Meath, Laois and Kilkenny areas, were identified where there were enthusiastic farmers, veterinary surgeons and dairy advisers. The participating veterinary surgeons were mentored on an ongoing basis over the course of a twelve month period, ensuring a consistency in approach and standards among them in dealing with mastitis at farm level. Approximately twenty herds were involved in the pilot project. Anticipated outcomes after 12 months include improvements in milk quality indices among participating herds, improved udder health and aspects of animal welfare relating to udder health on participating herds, increased awareness of the role of mastitis control in quality milk production, and increased capacity among participating veterinary surgeons in dealing with herd mastitis problems.

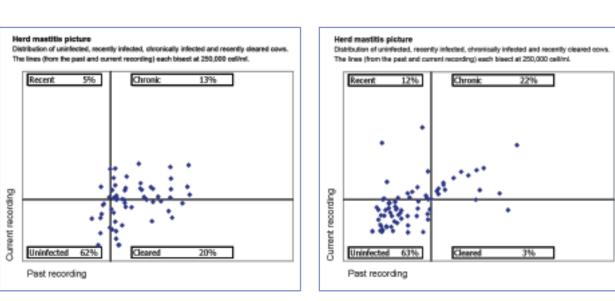


### Herd health reports

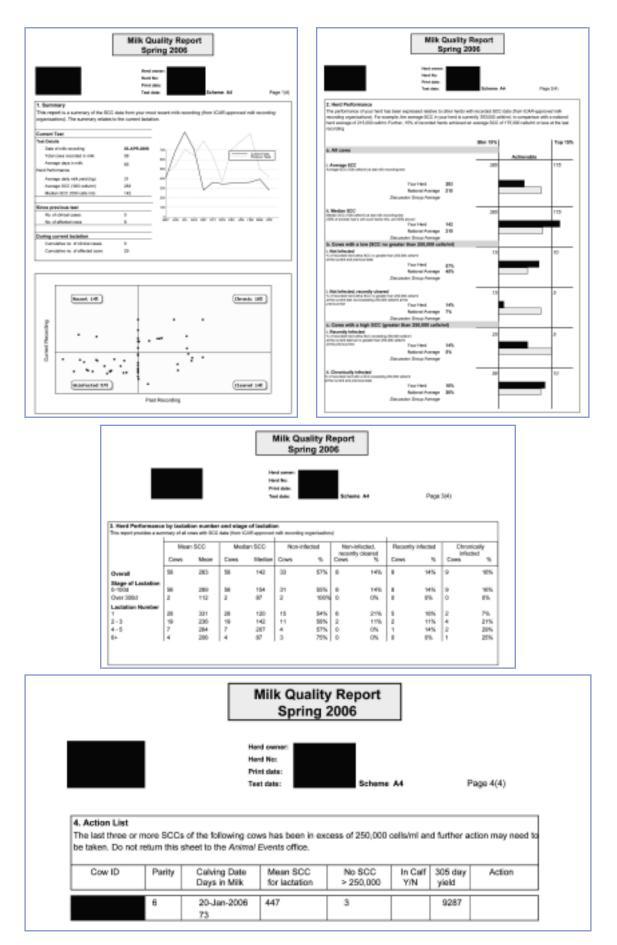
The objective of this project is the development of a series of herd reports based on key performance indictors of reproductive performance, milk quality, mortality and culling. The reports give an objective perspective of performance and can assist in decision making at farm level. Further, the reports enable farmers to compare themselves to their peers. They also provide tools in solving problems at farm level and monitoring progress following the implementation of control programmes. With time, the reports will be useful at national, regional and processor level to provide detailed information to assist with decision making.

The development of the series of reports is a joint initiative between the Irish Cattle Breeding Federation (ICBF) and CVERA, with a view to improving the sustainability and competitiveness of Irish cattle farming.





A summary of the mastitis picture on two Irish farms with a significant problem of Staphylococcal mastitis. On only one of these farms (left) is within-parlour transmission been controlled



Farmer milk quality reports developed collaboratively by the Irish Cattle Breeders Federation (ICBF) and CVERA

#### The infectious diseases pilot

Voluntary programmes of non-regulatory animal disease control are now common internationally. This pilot, which recently commenced, is seeking to build national capacity in three areas: voluntary programmes of animal health, the computing systems required to manage such programmes, and the laboratory resources that will be needed to manage relevant testing. In the pilot, a range of diseases to be considered include Johne's disease, infectious bovine rhinotracheitis (IBR), bovine viral diarrhoea (BVD), salmonellosis, neosporosis and leptospirosis. Voluntary programmes of non-regulatory animal disease control are now common internationally<sup>99</sup>

The economic impact of Johne's disease in an Irish dairy herd: A case study

Principal investigators: Margaret Good (DAF), Damien Barrett, Martin Hayes and Simon More (CVERA)

An epidemiological investigation, examining the economic impact of Johne's disease in an Irish dairy herd, concluded that infection was introduced into the herd in 1993 with the importation of 20 Dutch heifers. The practice of feeding pooled colostrum and milk was considered to have disseminated *Mycobacterium avium* subspecies *paratuberculosis* (MAP) widely throughout the herd. Farm performance declined substantially between 1993 and 2003, as a result of reduced milk yields, increased culling and reduced cull cow values. This negatively impacted on the profit margin per litre milk sold and per cow. The performance relative to a group of 25 to 30 peers also deteriorated over the study period. Farm performance was superior to that of its peer group until the late 1990s, but was markedly worse by 2002. Profit margin per cow had been  $\pounds 272$  greater than, but fell to  $\pounds 230$  less than, the group median in 2002. Similarly, when compared to the group median, average milk yield per cow was 814 (14.7%) litres above, but fell to 778 (13.9%) litres below in 2002.

Economic recovery commenced in 2003 as a result of the application of control measures that were applied from 2002 onwards.

<sup>66</sup>Following the introduction of Johne's disease onto the case farm, performance declined substantially over the following 10 years, as a result of reduced milk yields, increased culling and reduced cull cow values<sup>99</sup>

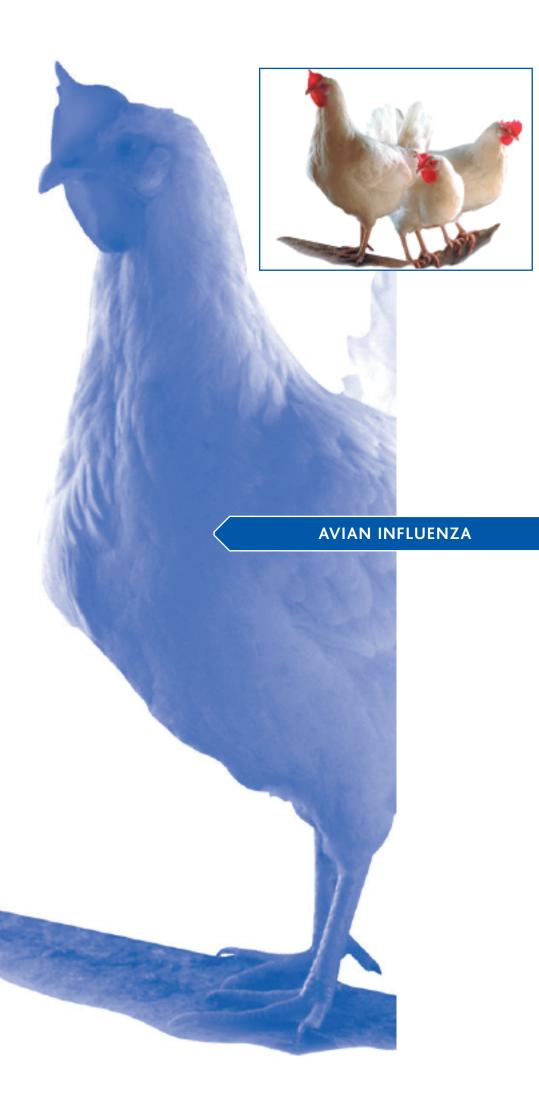
## Key meetings/presentations

#### Damien Barrett

- Workshop, national herd health initiative (Fermoy, 8-9 September 2004)
- Animal Health Committee, Veterinary Ireland (Dublin, 20 April 2004, 1 July 2004, 9 November 2004, 10 January 2005, 21 June 2005, 16 November 2005)
- Practitioner training, Veterinary Ireland (Abbeyleix, 16 September 2004, 2 December 2004)
- Series of meeting with ICBF personnel on developing herd health reports
- UCD seminar (Recent developments in herd health in Ireland) (3 March 2005)
- Multilateral meeting (industry/government) (Portlaoise, 25 April 2005).
   Annual Conference, Society for Veterinary Epidemiology and Preventive Medicine, Nairn (30 March – 1 April 2005)
- DAF presentation (On-farm records from ICBF) (Central Veterinary Laboratory, Abbotstown; 16 June 2005)
- 8th International Colloquium on Paratuberculosis, Copenhagen, Denmark (14-17 August 2005)
- Practitioner Discussion groups (Navan and Kilkenny, July, August, September, October, November 2005, January 2006)
- Industry meeting (Cork, 18 November 2005)
- Industry meeting (Kilkenny, 21 November 2005)
- Industry meeting (Longford, 16 December 2005)
- FSAI meeting (Dublin, 16 December 2005)
- Teagasc meeting (Dublin, 24 November 2005)
- Presentation on biosecurity for cattle herds (Laois/Offaly Friesian Breeders, Tullamore, 3 November 2005; Kildare Friesian Breeders, Naas, December 2005)
- Midland Clinical Society (Herd Health Recording Systems) (Mullingar, 10 November 2005)
- Practitioner and farmer meetings on herd health risk assessment (Moorepark, 2 December 2005; Grange, 5 December 2005)

Simon More

- Industry meeting (Mallow, 8 March 2004)
- Farmers meeting (Arklow, 1 April 2004)
- Animal Health Committee, Veterinary Ireland (Dublin, 20 April 2004, 1 July 2004, 9 November 2004, 10 January 2005, 21 June 2005, 16 November 2005)
  - UCD meeting (12 May 2004)
  - Industry meeting (Irish Farmers Association, Animal Health Committee; Abbeyleix, 26 August 2004)
- Workshop, national herd health initiative (Fermoy, 8-9 September 2004)
- Practitioner training, Veterinary Ireland (Abbeyleix, 16 September 2004)
- UCD meeting (2 November 2004)
- Practitioner training, DAF (Fermoy, 2 December 2004; Grange, 5 December 2004)
- Industry meeting (Kilkenny, 3 February 2005)
- UCD meeting (7 February 2005)
- Industry meeting (Irish Farmers Association; Dublin, 21 March 2005)
- Multilateral meeting (industry; government) (Portlaoise, 25 April 2005)
- UCD seminar (Opportunities for herd health in Ireland) (3 May 2005)
- Practitioner training, Veterinary Ireland/Association of Veterinary Surgeons Practicing in Northern Ireland (Enniskillen, 8 June 2005)
- Industry meeting (Irish Farmers Association; Dublin, 8 July 2005)
- Practitioner training, UCD Veterinary Hospital (26 August 2005)
- 8th International Colloquium on Paratuberculosis, Copenhagen, Denmark (14-17 August 2005)
- Practitioner discussion group (Kilkenny, 7 September 2005)
- Industry meeting (Kilkenny, 6 October 2005)
- Presentation, Joint AVSPNI (Association of Veterinary Surgeons Practicing in Northern Ireland)/CAVI (Cattle Association of Veterinary Ireland) conference (*The herd health initiative*) (Ballyconnell, 23 October 2005)
  - Industry meeting (ICMSA; Limerick, 7 November 2005)
- Presentation, Veterinary Ireland, Kerry Clinical Society (*The herd health initiative*) (Tralee, 15 November 2005)
- Industry meeting (Cork, 18 November 2005)
- Industry meeting (Kilkenny, 21 November 2005)
- Practitioner meeting, (Bandon, 14 December 2005)
- Industry meeting (Ballineen, 15 December 2005)
- Industry meeting (Longford, 16 December 2005)
- FSAI meeting (Dublin, 16 December 2005)
- Teagasc meeting (Dublin, 24 November 2005)



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## Overview

Principal investigators: Inmar Aznar, Simon More (CVERA), Olivia Crowe (BirdWatch Ireland) and John Wilson (National Parks and Wildlife Service)

nfluenza A viruses belong to the Orthomyxoviridae family and are widespread among wild birds and predominantly among waterfowl. Different subtypes have been described based on their haemagglutinin (15 H subtypes) and neuraminidase antigens (9 N subtypes). The H5N1 subtype was first isolated in Guangdong, China in 1996 causing the death of a moderate number of geese. Since then, the virus has been causing different outbreaks in the poultry industry. In 2002 the virus spread to humans in Hong Kong with a mortality rate of up to 50% of the infected population. Since late 2003, the H5N1 virus has expanded from a geographical and host range point of view. The spread of the virus to poultry and wild birds in Europe and Africa, together with the continuing H5N1 evolution and the increasing number of human infections, has raised concern about a possible influenza pandemic.

In recent years risk assessments have been used in the veterinary field as a method of evaluating risk resulting from a hazard. The risk assessment methodology (based on the OIE Animal Health Code) comprises three stages:

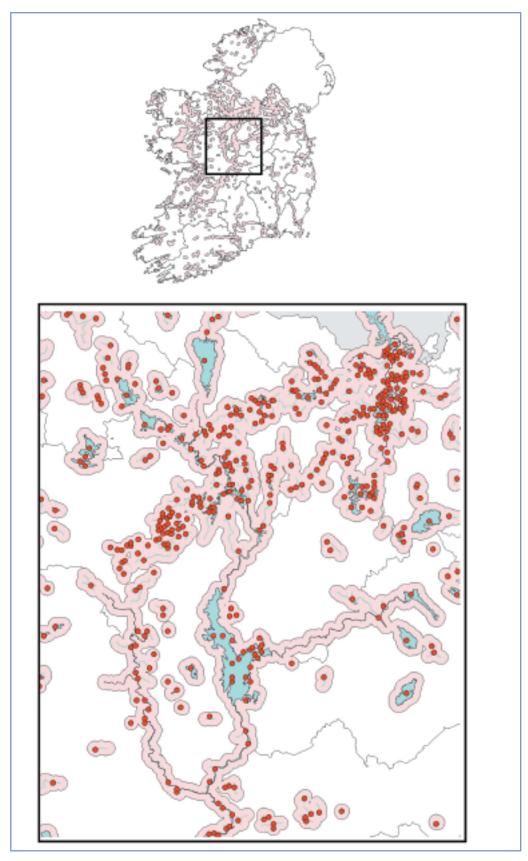
- Release assessment (the likelihood of entry, through the activities of wild waterbirds);
- Exposure assessment (the likelihood of spread to and within the commercial Irish poultry industry, following introduction); and
- Consequence assessment.

This methodology has been applied in order to qualitatively assess the likelihood that H5N1 virus will be introduced by wild waterbirds and will be spread to and within the Irish commercial poultry industry.

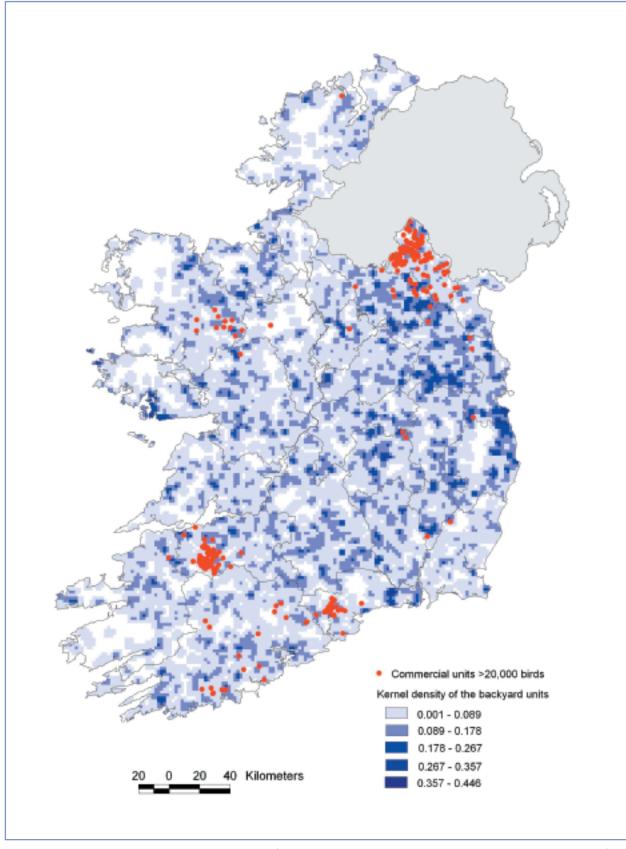
In collaboration with water-bird ecology experts, high risk migratory species wintering or breeding in Ireland have been identified. Migratory patterns and distribution in Ireland and Europe have been described for those species.

As part of the assessment, we are examining different ways in which H5N1 could spread among and between wild birds, commercial and non commercial units in the country. The Irish commercial poultry industry and related industry practices have been described in detail, and industry practices that could increase the risk of spread of the disease are currently being examined. Changes to high risk practices could lower the risk of introduction to and spread within the commercial industry, and may prove critical to industry survival if H5N1 infection were to become endemic in the Irish wild bird population. High-risk areas (based on species, enterprise type, density and proximity to wetlands and waterbirds) are being identified using the GIS software Arcview<sup>®</sup> 3.2, and a review of the biosecurity measures, surveillance strategies and the adequacy of existing statutory instruments is ongoing.

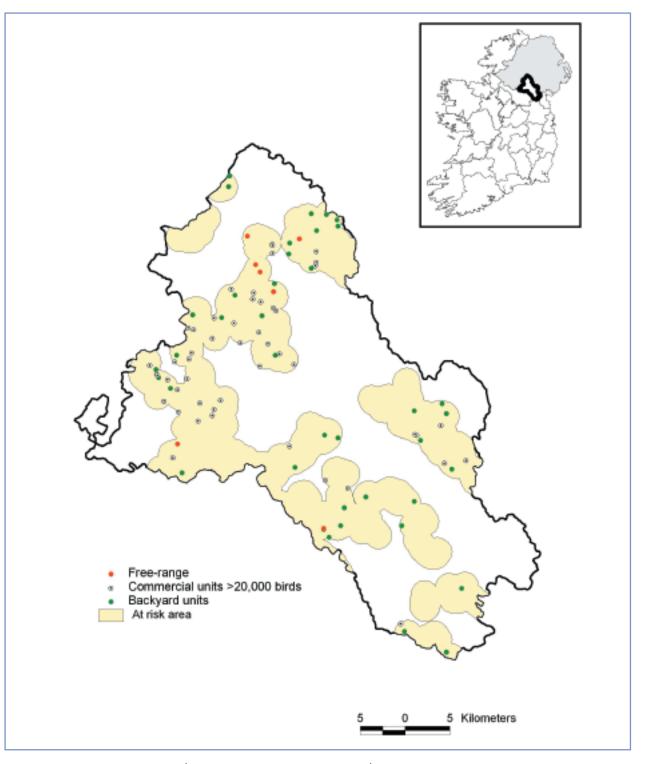
Work is underway to assess the likelihood that H5N1 will be introduced by wild waterbirds, and will be spread to and within the Irish commercial poultry industry<sup>99</sup>



High risk areas of Ireland, based on waterbird distribution. A 1.5 km buffer has been created around waterbodies (lakes in blue, rivers in black) and in which waterbird sampling sites (red dotes) occured



The distribution of commercial poultry units (each red dot represents a unit of greater than 20,000 birds) and backyard poultry (kernel density distribution, in blue)



Map of poultry units (free range, commercial, backyard) in high risk areas of Co. Monaghan

## Key meetings/presentations

Simon More
DAF meeting (Avian Influenza), Dublin (3 November 2005)



## GENERAL SUPPORT



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- 80 General mapping support

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- 81 The TB Testing Database
- 83 The Tracing Onward Tracking System (TOTS)
- 85 General database maintenance and interrogation

## Overview

CVERA provides general support in a wide range of areas, including epidemiology, statistics, geographical information systems (GIS) and database management.

## Epidemiological support

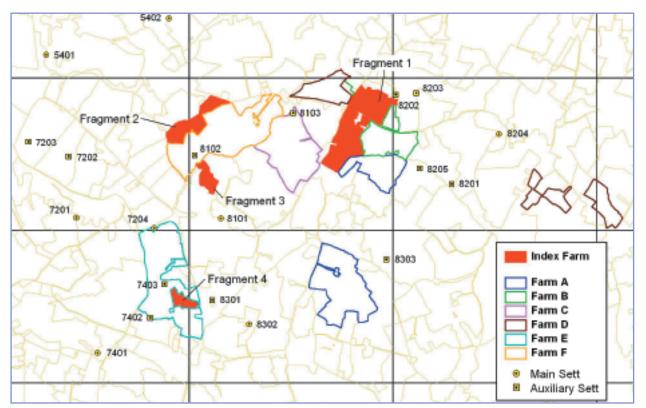
Key CVERA contact: Simon More

#### Farm investigations

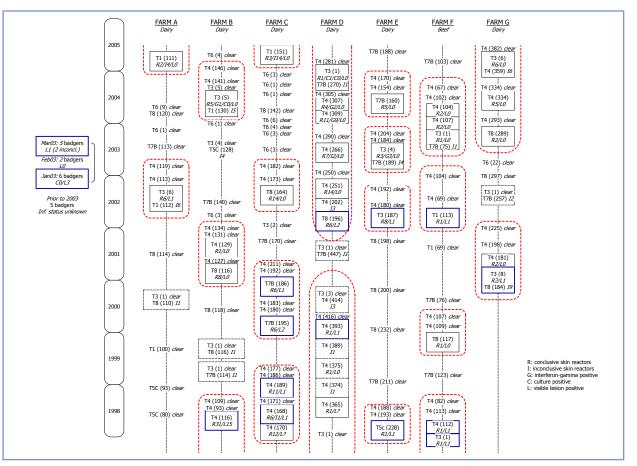
**F** arm investigations are a critical component of CVERA's work. These investigations offer the opportunity for CVERA staff to support veterinary students, and private government veterinarians to use practical epidemiological skills to solve (often complex) on-farm problems. Key epidemiological skills concern the use of simple methodologies to examine patterns of disease presentation in time, in space and among different animal groupings. Farm investigations are a key component of the curriculum for final year veterinary students. Further, these investigations are invariably conducted in collaboration with local veterinarians. The following investigations were conducted during 2004 - 2005:

- Illthrift (Co. Kilkenny, 14 April 2004, 22 October 2004, 22 February 2005)
- Mastitis (Co. Meath, 25 August 2004)
- Congenital abnormalities (Co. Louth, 30 September 2004)
- Poorly-defined illness in adult animals (Co. Meath, 7 October 2004, 24 November 2004)
- Mastitis, poor fertility (Co. Tipperary, 14 October 2004, 15 November 2004, 28 September 2005)
- Tuberculosis (Co. Monaghan, 28 October 2004)
- Johne's disease, mastitis, infertility (Co. Meath, 11 November 2004, 25 January 2005)
- Tuberculosis (Co. Monaghan, 1 February 2005)
- Tuberculosis (Co. Clare, 17 February 2005)
- Brucellosis (Co. Clare, 26 May 2005)
- Brucellosis (Co. Kerry, 22-23 June 2005)
- Tuberculosis (Co. Louth, 25 June 2005, 2 November 2005)
- Botulism (Co. Limerick, 20 September 2005)
- Tuberculosis (Co. Donegal, 12-13 October 2005)
- Tuberculosis (Co. Monaghan, 1 December 2005)
- Tuberculosis (Co. Wexford, 7 December 2005)

Simple epidemiological methods can be used in the field to examine patterns of disease in time, in space and among different animal groupings. This information then provides clues about the cause of the problem<sup>99</sup>



Farm investigation map for a tuberculosis breakdown in the south-east. The index farm has four fragments. Six of the contiguous farms (Farm A – F) were potentially significant in terms of source and/or spread



Farm investigation timeline for a tuberculosis cluster in the northeast. The period of each herd restriction is highlighted in red. The black and blue boxes indicate the presence of unconfirmed and confirmed reactors, respectively

## General epidemiological training

course in introductory epidemiology was held in Limerick (11-12 March 2004), Castlebar (18-19 March 2004) and Kilkenny (31 March-1 April 2004). The background to this course is clearly summarised in the email circulated to DAF staff in early 2004:

'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-anda-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 Veterinary Inspectors 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.'

The epidemiological mentoring group for veterinary inspectors has met on a number of occasions subsequently, at Athlone (27 May 2004) and Portlaoise (23 September 2004, 18 November 2004, 10 March 2005, 14 May 2005, 10 November 2005)

## North-south collaboration

he island of Ireland is essentially a single epidemiological unit. Consequently in the broad area of disease control, there would be mutual benefits for both Ireland and Northern Ireland from increased north-south collaboration. During 2004-2005, CVERA sought to contribute to north-south collaboration in the following situations:

- N-S working groups under the Good Friday Agreement, held on 28 September 2004, 11 March 2005, 11 October 2005
- N-S meeting to explore opportunities for cross-border projects, held at Ballymascanlon Hotel on 2 December 2004
- N-S brucellosis meeting, held in Newry on 22 December 2004
- The 4th International Conference on Mycobacterium bovis, held at Dublin Castle during 22-26 August 2005. The conference was organised by CVERA, in collaboration with the Department of Agriculture and Food in Dublin and the Department of Agriculture and Rural Development in Belfast
- Contributor to epidemiological training for DARD Veterinary Officers (9 and 17 November 2005)

## General epidemiological support

uring 2004/05, CVERA contributed to a range of additional epidemiological support activities, as follows:

- External referee, Wildlife Epidemiology course within the Masters of Veterinary Public Health Management, University of Sydney (2004)
- External examiner, MSC (Veterinary Epidemiology; internal students), Royal Veterinary College (2004, 2005)
- DAF workshop (food safety), University College Dublin (10 September 2004)
- Inaugural professorial lecture, University College Dublin (21 October 2004)
- DAF workshop (surveillance), Newbridge (5 November 2004)
- DAF workshop (meat inspection), University College Dublin (26 April 2005)

•• Epidemiology is a valuable skill for front line veterinarians<sup>99</sup>

••There would be mutual benefits for both Ireland and Northern Ireland from increased north-south collaboration ""

GENERAL SUPPOR

## Statistical support

Key CVERA contact: Tracy Clegg

uring 2004-2005, CVERA provided statistical support and advice to a range of researchers and projects, as follows:

- Central Veterinary Laboratory, Department of Agriculture and Food, Abbotstown: A study of helminth parasites in culled cows from Ireland.
- UCD School of Agriculture, Food Science & Veterinary Medicine, University College Dublin: A study of dry cow therapy and effects on SCC in 10 Irish dairy herds.
- Central Veterinary Laboratory, Department of Agriculture and Food, Abbotstown: Effect of Neospora caninum sero-positivity on milk production in an endemically infected dairy herd.
- Department of Agriculture: Comparison of the potency of different tuberculins used in the field compared to the Irish standard.
- *Vet-Aqua International and the Marine Institute, Galway*: Epidemiology of Pancreas disease amongst atlantic farmed salmon in Ireland.
- Department of Agriculture: A study into the age of animals slaughtered at knackeries.
- University College Dublin Veterinary Hospital, School of Agriculture, Food Science & Veterinary Medicine, UCD: The influence of sternal vs. lateral recumbency on the L5-L6 mid-laminar distance amongst dogs.
- UCD School of Agriculture, Food Science & Veterinary Medicine, University College Dublin: Non-accidental injury in companion animals.

## **GIS** support

Key CVERA contacts: Guy McGrath and Dan Collins

## The Wildlife Unit

**C**VERA act as an independent monitor for the Department of the Environment's Parks and Wildlife Services to ensure operations of the Department of Agriculture's Wildlife Unit 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 Department of the Environment and the Department of Agriculture.

#### Administration of the Wildlife Unit

n 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 badgers setts found through surveying are then digitised and maintained centrally on the GIS.

CVERA provided independent statistical support and advice to a range of researchers and projects

•• CVERA maintain the GIS component of the Wildlife Unit administration centre

#### 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 emergence of a Class A disease

## Database support

## The TB Testing Database

Key CVERA contacts: Paul White and Isabella Higgins

#### Introduction

Since the introduction of the Animal Health Computer System (AHCS) and other online computer systems within the Dept of Agriculture, increasing volumes of data have accumulated in relation to animal disease and movement within the Irish cattle herd. Recent advances in PC hardware/software have made it possible to use such datasets for research. This process requires a familiarity with current database management tools, as well as an understanding of data acquisition procedures.

#### Development

The CVERA national tuberculosis/brucellosis testing database project has been an ongoing process since 1998. It has continued to play a supportive role in the research programme with the original aim of providing a central database for querying of tuberculosis/brucellosis testing data from the 29 District Veterinary Offices (DVOs). The database was initiated on Microsoft Access<sup>™</sup> which provided-user-friendly interface for running queries about the tuberculosis/brucellosis Eradication schemes in relation to:

- Tuberculosis test summary data
- · Tuberculosis reactor and inconclusive skin results
- · Tuberculosis post-mortem results for reactor animals
- · Contiguous herds identified by DAF field staff
- Brucellosis test summary data

In early 2005, the Dept of Agriculture completed the rollout of the AHCS system replacing the earlier Nixdorf system as a tool for management of the tuberculosis/brucellosis testing schemes. The shadow database held within CVERA was upgraded to coincide with the introduction of AHCS. Microsoft SQL Server<sup>™</sup> was chosen as a platform for reasons of data security, flexibility and scalability to handle larger datasets.

The migration process required that existing (Nixdorf) data be reformatted to conform to changes in database architecture introduced under AHCS. Migrated data was merged with recently extracted data from AHCS to form a seamless dataset extending throughout the period 1989 to date. Data management within CVERA continues to be a dynamic process with its scale and complexity driven by the ongoing demand for new datasets to be explored within the research programme. Recently, the system has been expanded to deal with laboratory results for TB suspect lesions as they are become available through AHCS.

Data management within CVERA continues to be a dynamic process with its scale and complexity driven by the ongoing demand for new datasets to be explored within the research programme<sup>99</sup>

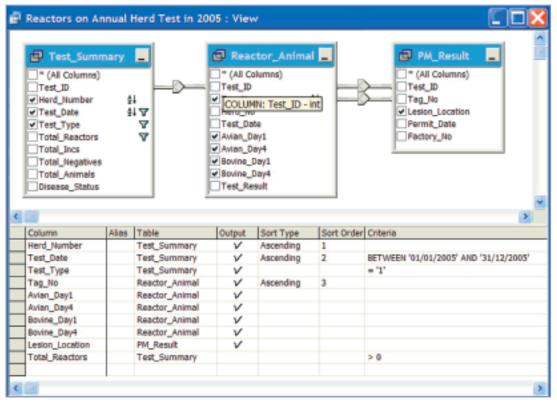
CVERA provide a broad range of mapping support<sup>99</sup>

#### Management

The database is updated at monthly intervals by running a AHCS report that outputs data to standardised text files. The text files are uploaded onto the SQL server database using an access front-end to automate various server stored procedures. Before new data is added to the system, a series of validation checks are done to ensure continuity of data, remove duplicate records, amalgamate part test records, deal with re-interpreted tests, and ensure internal consistency between related tables. The system is now fully up-to-date and currently holds in excess of 4 million tuberculosis herd test records recorded along with associated skin test readings and post-mortem results.

#### Interrogation

The ability to run Structured Query Language (SQL) is a key feature of modern relational database management systems that enables questions can be asked about data stored across various tables. By running queries to combine data derived from disparate sources, the system offers the potential to utilise animal movement data to study animal disease data. Because large tables are involved, this process is resource intensive and calls for carefully planned query design. The following figure illustrates the query-by-example grid provided for running basic queries from an MS Access™ front-end application.



Screenshot of a database query

In dealing with tuberculin testing data over long periods, Collins (1995) warns that definition of a "reactor" has not remained constant over time. Variations in the frequency of testing, together with the types of tuberculins and the degree of severity of interpretation of the test results, may have had a direct effect on the apparent incidence and prevalence of the disease as measured by means of the tuberculin test (Collins, 1995).

The database does not provide information on all of the factors that govern the application of the tuberculin skin test. Apart from skin test readings for the animal/herd, animals may be deemed reactor in light of factors such as ancillary blood tests, or for other epidemiological reasons. Likewise, the finding of one of more animal(s) with a confirmed factory lesion foreshadows a restriction in the herd from

which the animal originated. Such factors in addition to the previous tuberculosis testing history of the herd or animal and the TB testing history of neighbouring herds are among the key considerations that influence the test interpretation and subsequent follow-up in the DVO.

#### References

Collins, J.D., 1995. In: '*Mycobacterium bovis* Infections in Animals and Humans' (Thoen, C.O. and Steele, J.H., eds), Iowa State University Press, Ames, pp. 224-238.

#### The Tracing Onward Tracking System (TOTS)

Key CVERA contacts: Paul White and James O'Keeffe

#### Development

The Tracing Onward Tracking System (TOTS) was developed as an aid to the management and tracking of animals originating from tuberculosis/brucellosis and BSE infected herds which move to other herds where they may pose a disease risk. These fall into the following categories:

- Animals originating from High Risk tuberculosis infected herds;
- Animals originating from High Risk brucellosis infected herds;
- Animals originating from tuberculosis derogated herds (i.e. herds which were free to trade within the state with an inconclusive animal that subsequently tested reactor);
- BSE cohort animals; and
- BSE progeny animals.

The tracing procedure commences with the recording of a breakdown within an index herd for which a tuberculosis/brucellosis epidemiology visit is assigned to a Veterinary Inspector. During this visit, at-risk animals are identified for logging on the TOTS system for tracing nationwide.

Each index herd may have multiple animals identified for onward tracing. The form for recording these is shown on the animal tracing form.

Based on an animal's last known location, the system sends the tracing details over the network to the DVO responsible for completing the tracing

Animal for Br Onward Tracing - Meath DVO	
Ref. No.: 69 Tag Number: IE281	073421529 🖹 Risk: Higher 🔽
Animal status: Awaiting tracing	Animal Type: Cow 🔽
Comments:	×
Index Herd Animal Location Documents	
Previous Location	Nest Location:
Location Type: Herd 🔽	Location Type: Herd
Location No: Q1010019	Location No: A101001X
Name: MR A. FARMER	Name: MR P. MURPHY
Address: HILL FARM	Address: MEADOW FARM
NORTHLANDS ANY TOWN	THIS TOWN CO CARLOW
CO. MEATH	
Phone/Mobile:	Phone/Mobile:
Record Movement	Date Moved: 22/11/2005
	<u>Q</u> K <u>Cancel</u>

Screenshot of an animal tracing form

Based on an animal's last known location, the system sends the tracing details over the network to the DVO responsible for completing the tracing. Within each DVO, a list of work in progress is maintained, and this is checked on a regular basis. The system prints any follow-up documentation required and provides for the recording of the outcome in respect of each animal completed.

In addition to the core tracing functions, the system also logs contiguity visits for tuberculosis, Wildlife Unit survey requests and the allocation of wildlife unit surveys to TAO field staff. Reporting options are available for these activities.

Following a period of end user training, the TOTS System was deployed to all DVO's in October 2003 in the form an access database located on each DVO file server, with an access front-end installed on each user PC. The system processes approximately 20,000 at-risk animals each, of which 75% are for tuberculosis tracing.

#### Management

Some ongoing servicing of queries has been required due to the multiplicity of locations and users and because of the volume of work processed by the system. Since the initial release, there have been ongoing requests for modifications and upgrades requiring impact assessments, software programming, documentation, system testing, deployment and end-user training.

A number of enhancements were deployed to the system during 2005 which included:

- A facility to allocate epidemiology visits to VIs in relation to brucellosis-restricted herds;
- A facility to allocate contiguity visits to TAOs in relation to tuberculosis-restricted herds;
- BSE Cohort/Progeny tracing: enhancements to a BSE6, BSE 4e reports;
- Enhanced central management reporting including the reporting of onward tracing at the herd or animal level both regionally and by DVO;
- A facility for VI's to approve the list of animals for BSE tracing before sending to other DVOs;
- Improved integration with AHCS to allow rapid searching of animals by tag number;
- Validation to ensure that key criteria are met before processing a request for Wildlife Unit survey; and
- The survey progress report has had additional columns and selection criteria added to facilitate reporting by breakdown date.

#### Interrogation

From a central location, a series of reports can be run both at animal and herd level to summarise progress by to date both regionally and by District Veterinary Office. A facility also exists to extract the key datasets to delimited text files for management and research purposes.

#### General database maintenance and interrogation

#### Key CVERA contact: Isabella Higgins

CVERA maintains the badger post-mortem database and delivers data to CVERA and other researchers following the interrogation of each of the following national databases:

- AHCS database
- CMMS database
- Factory surveillance database
- LIMS database
- AHCS database
- TOTS & RHMS
- ER76 database

The following examples illustrate how these data are subsequently used:

a. The provision of data for ongoing work, PhD theses and various papers, including:

- A long term study of the impact of proactive badger removal on herd restrictions due to bovine TB in east Offaly 1989-2004
- The genetics of predisposition to tuberculosis in Irish dairy and beef cattle.
- Tuberculin registration (frequency of testing)
- APT figures on a DED basis for production of thematic maps

b. Data from the east Offaly badger post-mortem database (1997-2003) was used to reconcile badger licenses from the control area of the east Offaly project during these years.

c. Data from the calf birth registration database was used to assess the accuracy of individual animal identification. In this study, the DNA profile of the calf was compared with the profile of the registered dam to determine if the dam qualifies as a parent of the calf through DNA testing.

d. Data relating to tuberculin tests carried out on cattle and the number of tuberculin reactors disclosed according to county were compiled to produce the Bovine Tuberculosis Statistics, Annual Summary (2003-2004).

e. These databases were also used to support the design of questionnaires. For example, the National Bovine Leptospirosis study was conducted to determine the seroprevalence of leptospirosis in unvaccinated suckler / beef herds in the Republic of Ireland and the associated risk factors for disease. The study involves two parts:

- a questionnaire to be sent to farmers randomly chosen from the national herd database; and
- serology (ELISA) on sera already available from the last herd test (2004 / 2005).

# 66 Database

interrogation plays an important role in the support of a number of national projects<sup>99</sup> BIENNIAL REPORT 2004/2005



## SELECTED PAPERS



## Improved statistical measures for TB surveillance and control

I.M. Higgins, S.J. More, T.A. Clegg and P.W. White

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#### Abstract

Objective measures of progress are a critical component of any effective disease eradication programme. As part of a tuberculosis eradication programme, it is important that these measures are based on herd as the unit of interest. Also, they should clearly distinguish the results of surveillance and control activities, and use clearly defined case definitions. In Ireland, new statistical measures were developed to assist with decision-making, both locally and at a national level. Measures relating to surveillance and control activities were used, after creating an 'episode' file which defined periods when each herd was (and was not) restricted due to tuberculosis. During 2005, 94.7% of eligible herds remained disease-free during the year. There was minimal correlation between APT (an existing measure; the reactor disclosure rate per 1,000 animal tests performed) and herd incidence (Spearman's rank correlation = 0.216, p = 0.251). Based on herds restricted on 31 December 2005, there was an average of 5.7 reactors per restriction, with a single reactor detected in 41.2% of restrictions. These herd-level measures effectively partition activities relating to detection of new cases (surveillance) and the resolution of cases following detection (control). They also support earlier findings about herd-level risk factors for herd TB breakdowns in Ireland. Further work is ongoing.

#### 1. Introduction

Objective measures of progress are a critical component of any effective disease eradication programme. This issue was discussed in some detail at the 4th International Conference on *Mycobacterium bovis* (Anon., 2006), drawing on the experience from a range of countries. Previously, there had been a similar discussion on this topic within Ireland (More, unpublished). Key questions under discussion included 'how the level of tuberculosis was measured?', 'how progress was defined?', and 'how targets were set?' Despite divergent national approaches, at these two meetings there was general agreement on a range of relevant issues including the use of herd as the unit of interest, the need to clearly distinguish the results of surveillance and control activities, and the use of clearly defined case definitions.

For many years, the APT (the reactor disclosure rate per 1,000 animal tests performed) has been the primary measure of progress within the Irish programme. An APT of 2.86 was achieved nationally during 2005. As noted previously, there are a range of difficulties with the APT, including the influence of testing intensity on this measure (as the intensity of testing increases, the denominator is likely to increase more quickly than the numerator). Further, APT includes results from both surveillance and control activities, and focuses on the animal rather than the herd (Griffin *et al.*, 2005).

In recent years, there have been substantial advances in the analysis and management of the national tuberculosis database in Ireland. The national Animal Health Computer System (AHCS), which was introduced in 1986, has recently been upgraded, and facilitates the central collection and management of national data. This now provides almost 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 the above-mentioned recommendations. In this paper, we report early results from this work.

#### 2. Materials and methods

Data

For this study, we queried AHCS (Animal Health Computer System), which records summary (herd TB testing) data on national TB control activities from 1989 to the present. These records were used to create an episode file, defining periods when each herd was (and was not) restricted due to tuberculosis. A visual illustration of TB episodes is presented in Figure 1. In the episode file, each record defines a unique restriction episode (therefore, a single herd with three episodes will have three separate records). Herds without cattle were excluded from the episode file. Each episode was associated with the relevant classifying variable (for example, DVO, herd size etc).

These calculations include herds restricted following factory surveillance (detection of lesion(s) in animals from attested herds at slaughter) and (tuberculin testing). However, if a restriction was triggered by a factory lesion, for computational reasons this animal(s) was not counted as a reactor during the restriction.

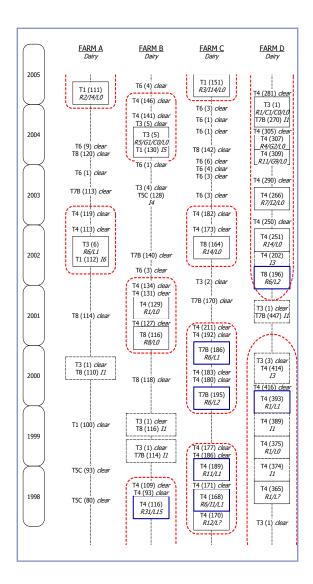


Figure 1. A timeline of tuberculosis-related events between 1998 and mid-2005 on 4 farms in Co. Louth. Each period of herd restriction ('an episode') is highlighted in red. The testing history is presented in abbreviated form (R: conclusive skin reactors; I: inconclusive skin reactors; G: reactors to the interferon- $\gamma$  test; C: culture positive; L: TB lesion detected at slaughter). Therefore, *T1 (110) R1* indicates that test type 1 (the annual round test) was conducted on 110 animals, with one conclusive reactor. The boxes are indicative of confirmed reactors (blue box), conclusive but not confirmed reactors (solid black) and inconclusive reactors (dashed black)

#### Measures of progress

Two measures of surveillance activity were calculated, each focusing on the 12 month period from 1 January 2005. In each case, calculations were restricted to active herds (those tested for TB at least once during the 15 months prior to 1 January 2005):

- The herd disease incidence (the number of active herds that became restricted during the period/the total number of active herds that were unrestricted on 1 January 2005). If herds were restricted on more than one occasion during 2005, only the first was considered; and
- The percentage of herds remaining disease free (the number of active herds that remained unrestricted during the period/the total number of active herds that were unrestricted on 1 January 2005).

These measures were calculated for all active herds, and classified by District Veterinary Office, geographic disease risk (a measure of local disease chronicity; using data from 2000 to 2005, the number of years that the APT in each District Electoral Division had been 2.5 or greater; Figure 2), herd size (the average of all full-herd tests during 2005, or the preceding year if no testing occurred in 2005) and production type (dairy, beef, other, suckler).

Seven control measures were calculated, for all Irish herds restricted due to TB on 31 December 2005:

- Duration of restriction (in days);
- Number of reactors per restriction;
- % singleton breakdowns (percentage of currently-restricted herds where only a single reactor tuberculin test and/or factory surveillance had been detected during the restriction);
- % 3rd reactor-retest (percentage of restricted herds where a 3rd reactor-retest has been conducted);
- % 4th reactor-retest (percentage of restricted herds where a 4th reactor-retest had been conducted);
- Inter-episode interval (days between the start of the current and the end of the previous restriction; only calculated for herds with a
  previous episode); and
- · Repeat restrictions during the preceding 3 years.

These measures were also classified, as previously.

For continuous variables, we calculated the mean, minimum, 25th percentile, median, 75th percentile, maximum; for categorical variables, we calculated percentages. The correlation between APT and herd incidence was undertaken using Spearman's rank correlation.

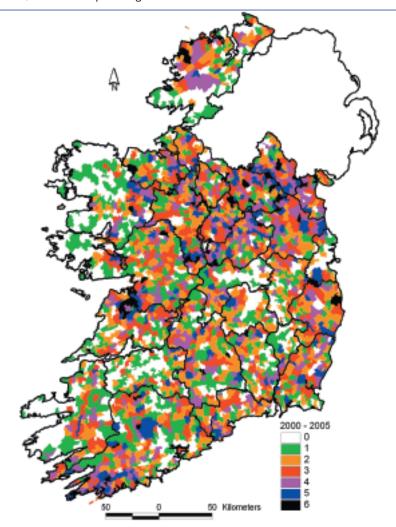


Figure 2. Historic disease risk, by District Electoral Division (DED). This assessment was determined according to the number of years that the APT (the reactor disclosure rate per 1,000 animal tests performed) in each DED had been 2.5 or greater

## 3. Results

#### Surveillance

During 2005, the herd incidence was 5.3% (6,285 restrictions, 117,823 herds on 1 January 2005). Therefore, 94.7% of the eligible herds remained disease-free during the year. Herd disease incidence was highest in West Cork (10.2%), Waterford (9.3%) and Westmeath (9.1%), and lowest in Leitrim (3.4%), Kerry (2.6%) and Mayo (1.8%) (Table 1). The surveillance measures, by geographic disease risk, herd size and production type, are presented in Table 2.

Table 1	I. Tuberculosis s during 2005	urveillance measures , by county
	Herd disease	% herds remaining
	incidence (%)	disease-free
	. ,	
Total	5.3	94.7
County Carlow	6.8	93.2
Cavan	5.2	94.8
Clare	4.3	95.7
Cork Central	6.2	93.8
Cork North	7.5	92.5
Cork West	10.2	89.8
Donegal	3.9	96.1
Dublin	7.8	92.2
Galway	4.4	95.6
Kerry	2.6	97.5
Kildare	4.6	95.4
Kilkenny	8.0	92.0
Laois	6.3	93.7
Leitrim Limerick	3.4 4.0	96.6 96.0
Longford	4.0 7.8	90.0
Louth	6.6	93.5
Mayo	1.8	98.2
Meath	8.7	91.3
Monaghan	4.7	95.3
Offaly	6.0	94.0
Roscommon	4.3	95.7
Sligo	4.3	95.8
Tipperary No		92.9
Tipperary Sou		92.5
Waterford	9.3	90.7
Westmeath	9.1	90.9
Wexford Wicklow East	8.0 6.3	92.0 93.7
Wicklow Wes		93.7 94.5
WICKLOW Wes	L J.J	54.5

Table 2. Tube	erculosis surveillanc geographic disease and product	
	Herd disease incidence (%)	% herds remaining disease-free
Total	5.3	94.7
Geographic di	sease	
riskª		
0	2.5	97.5
1	4.4	95.6
2	5.7	94.3
3	5.8	94.2
4	7.7	92.3
5	8.5	91.5
6	9.7	90.3
Herd size		
< 25	2.1	97.9
25-49	4.5	95.5
50-74	5.8	94.2
75-99	8.2	91.8
100+	11.9	88.1
Production typ	be	
Beef	4.6	95.4
Dairy	8.2	91.8
Other	5.5	94.6
Suckler	4.5	95.5

a Determined according to the number of years that the APT (the reactor disclosure rate per 1,000 animal tests performed) in each DED had been 2.5 or greater

There was minimal correlation between APT and herd incidence measured in each District Electoral Division during 2005 (Spearman's rank correlation = 0.216, p = 0.251; Figure 3).

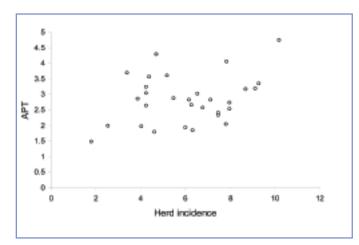


Figure 3. Relationship between APT (the reactor disclosure rate per 1,000 animal tests performed) and herd incidence in each District Electoral Division during 2005

#### Control

On 31 December 2005, 3,689 herds were restricted due to tuberculosis. On average, there were 5.7 (25th percentile 1, median 2, 75th percentile 6) reactors per restriction; in 1,519 (41.2%) restrictions, only one reactor was detected. Control measures relating to the current restriction and the period prior to the current restriction are presented in Tables 3-6.

		ion of on (days)		ber of ctors	Singleton breakdowns	3rd reactor retest	4th reactor retest
	Mean	Median	Mean	Median	(%)	(%)	(%)
Total	149.8	112	5.7	2	41.2	13.0	4.7
County							
Carlow	130.6	93	5.2	1	61.2	6.1	4.1
Cavan	166.7	114	7.4	2	38.1	17.4	7.7
Clare	127.0	109	7.2	2	36.8	10.3	1.3
Cork Central	154.7	133	7.3	3	30.8	19.2	5.4
Cork North	133.1	99	5.6	2	38.3	10.5	3.1
Cork West	140.8	120	5.4	2	35.3	15.8	3.8
Donegal	135.7	113	3.9	1	51.3	9.0	1.3
Dublin	203.1	134	1.9	1	42.9	14.3	4.8
Galway	156.6	110	5.6	2	39.5	14.7	3.3
Kerry	103.4	86	5.3	2	45.9	6.6	0.8
Kildare	251.9	107	7.4	2	44.8	22.4	17.2
Kilkenny	180.3	142	5.6	2	41.2	20.9	5.9
Laois	147.0	114	3.9	1	53.7	12.6	5.3
Leitrim	150.3	134	5.3	3	37.9	13.8	1.7
Limerick	124.0	95	6.3	2	41.3	8.4	2.1
Longford	106.1	92	4.8	2	36.4	6.8	1.7
Louth	144.9	120	6.4	2	40.0	11.4	5.7
Mayo	141.7	110	4.0	1	61.4	10.2	3.4
Meath	191.3	133	5.4	2	41.1	13.6	7.5
Monaghan	136.1	103	9.8	4	33.3	10.1	4.0
Offaly	175.0	112	4.0	1	49.6	13.3	7.1
Roscommon	123.6	100	4.8	2	43.2	18.2	5.3
Sligo	120.2	93	3.7	2	42.1	9.2	1.3
Tipperary North	165.6	100	8.1	3	40.8	14.3	9.5
Tipperary South	141.3	110	5.3	3	33.6	11.2	5.9
Waterford	136.9	113	6.8	2	37.6	12.8	5.5
Westmeath	148.0	109	5.6	3	34.9	14.4	6.2
Wexford	177.3	127	4.2	1	47.5	10.1	5.6
Wicklow East	167.8	114	4.6	2	49.0	8.2	6.1
Wicklow West	192.8	91	6.2	2	40.0	20.0	0.0

Table 4. Control measures relating to the current restriction, for herds restricted on 31 December 2005,

		tion of on (days)		ber of ctors	Singleton breakdowns	3rd reactor retest	4th reactor retest
	Mean	Median	Mean	Median	(%)	(%)	(%)
Total	149.8	112	5.7	2	41.2	13.0	4.7
Geographic dis	sease risk°						
0	132.0	105	1.7	1	65.2	5.8	2.2
1	131.4	100	3.9	1	50.2	9.2	2.7
2	148.3	113	5.4	2	41.6	11.5	4.1
3	148.9	107	5.7	2	39.1	14.4	5.9
4	159.4	120	6.3	3	35.8	15.7	5.4
5	163.5	124	8.4	3	29.2	15.3	5.5
6	182.3	113	9.7	5	26.0	23.2	9.6
Herd size							
< 25	167.3	119	2.6	1	56.9	6.8	1.2
25-49	130.1	106	3.6	1	49.3	10.9	2.4
50-74	130.4	112	5.1	2	40.6	9.4	3.2
75-99	128.6	106	5.1	2	41.1	13.8	4.2
100+	165.7	114	8.3	3	30.9	17.6	8.0
Production typ	be						
Beef	174.5	118	3.0	1	55.8	11.8	4.9
Dairy	146.8	114	7.7	3	31.6	14.7	5.7
Other	119.4	92	3.0	1	51.7	6.9	0.0
Suckler	143.1	106	5.1	2	43.0	12.2	3.9

each DED had been 2.5 or greater

		asures relating to the period ds restricted on 31 Decembe		on,
		ode interval ays)ª		estrictions eceding 3 years)
	Mean	Median	Mean	Median
Total	864.7	748.5	0.35	0
County				
Carlow	1044.0	1025.0	0.31	0
Cavan	799.5	672.5	0.62	0
Clare	879.5	917.0	0.28	0
Cork Central	1014.1	1080.5	0.31	0
Cork North	785.5	571.0	0.39	0
Cork West	716.1	582.0	0.45	0
Donegal	918.9	858.0	0.13	0
Dublin	1170.0	749.0	0.24	0
Galway	878.3	832.0	0.21	0
Kerry	1072.2	939.5	0.28	0
Kildare	1034.7	931.0	0.36	0
Kilkenny	977.4	921.0	0.39	0
Laois	616.7	599.5	0.31	0
Leitrim	1126.9	1048.0	0.19	0
Limerick	1342.4	1678.0	0.20	0
Longford	866.7	853.0	0.34	0
Louth	1002.7	932.0	0.34	0
Mayo	878.3	670.5	0.23	0
Meath	793.3	659.0	0.40	0
Monaghan	944.0	913.5	0.35	0
Offaly	729.1	693.0	0.39	0
Roscommon	734.3	681.0	0.35	0
Sligo	973.8	896.0	0.22	0
Tipperary North	926.2	937.5	0.35	0
Tipperary South	864.6	640.0	0.33	0
Waterford	937.3	958.5	0.39	0
Westmeath	805.2	784.0	0.44	0
Wexford	754.5	596.0	0.48	0
Wicklow East	452.3	253.0	0.71	1
Wicklow West	338.0	338.0	0.70	0.5

Table 6. Control measures rel	ating to the period prior to the current restriction, for herds restricted on 31 December 2005,
	by geographic disease risk, herd size and production type

	· · · · · · · · · · · · · · · · · · ·	ode interval ıys)°	•	estrictions eceding 3 years)
	Mean	Median	Mean	Median
Total	864.7	748.5	0.35	0
Geographic disease	e risk <sup>₅</sup>			
0	920.8	879	0.22	0
1	928.7	902	0.25	0
2	852.3	728	0.31	0
3	867.6	710	0.36	0
4	893.0	829	0.38	0
5	846.7	724	0.46	0
6	640.5	484	0.61	0
Herd size				
< 25	914.6	824	0.16	0
25-49	970.0	899	0.23	0
50-74	1017.8	976	0.27	0
75-99	916.3	911	0.33	0
100+	763.0	594	0.52	0
Production type				
Beef	825.8	679	0.30	0
Dairy	833.0	672	0.44	0
Other	1076.2	967	0.28	0
Suckler	913.7	824	0.30	0

<sup>a</sup> Only calculated for herds with a previous episode
 <sup>b</sup> Determined according to the number of years that the APT (the reactor disclosure rate per 1,000 animal tests performed) in each DED had been 2.5 or greater

#### 4. Discussion

A range of measures were calculated during this study, to accurately reflect progress within the national TB eradication programme. These herd-level measures effectively partition activities relating to detection of new cases (surveillance) and the resolution of cases following detection (control). This information, provided on an ongoing and timely basis, should be of benefit to national and regional programme decision-makers. As expected, there was only limited agreement between herd incidence and APT, noting that the former is a measure of surveillance whereas the latter measures both surveillance and control (Figure 3).

These results support earlier findings about herd-level risk factors for herd TB breakdowns in Ireland. In addition, these results provide preliminary insights (based on univariate analysis) into the varying effects of these risk factors on disease presence (surveillance) and resolution (control). The spatially clustered nature of this disease in Ireland, as highlighted in Figure 2, is well-described (More and Good, 2006). Based on the current results, areas of historic disease risk tend – on average – to have more outbreaks (higher herd incidence, shorter inter-episode interval, a greater number of repeat restrictions), with these outbreaks being more-severe (more reactors, fewer singleton breakdowns) and taking longer to clear (longer periods of restriction, a higher percentage of 3rd and 4th reactor retests). However, because geographic disease risk was calculated based on APT, these preliminary results need to be interpreted with care. APT is a combined measure of surveillance and control, which may explain the above-mentioned associations. In addition, there is the potential for error when APT is calculated for small geographic areas (such as DEDs throughout Ireland), with varying and often small numbers of cattle, and in situations where there is precautionary removal of animals. Relevant to this latter case, APT includes all (and not just conclusive) reactors (including, for example, in-contacts). Alternative measures of geographic disease risk are currently being considered. Increased herd size is similarly associated with increased disease risk, as noted previously by a range of authors including Olea-Popelka et al. (2004). The longer period of herd restriction in small herds (<25 cattle) is at odds with this general trend (Table 4), for reasons that are currently not apparent. During 2005, herd incidence on dairy farms was higher in comparison with beef, suckler or other production types. Although these outbreaks were larger and more frequent, they also cleared more quickly.

Further work is ongoing. In particular, multivariate analyses will be conducted to determine the relative importance of the abovementioned risk factors. Work will also be undertaken to critically evaluate the association between surveillance and control.

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# An assessment of injury to badgers due to capture in stopped restraints

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#### Abstract

Badgers from culling operations carried out by the Department of Agriculture and Food (DAF) between October and December 2005 were examined at post-mortem to determine the frequency and severity of injuries occurring when badgers are captured using stopped restraints. Skin damage and damage to the underlying tissues caused by the restraint were classified following visual examination. Of the 198 badgers examined, 94.6% had either no skin trauma or minor abrasions, 76.9% had no or localised subcutaneous tissue damage, while 98.5% had either no muscle damage or slight bruising as a result of the restraints. Of those examined 2% had cuts to the skin, 1.5% had extensive subcutaneous oedema, while 1.5% had areas of haemorrhage and tearing of muscle. Four (2.0%) badgers had injuries considered clinically significant. The data indicates that the stopped restraint as used in the Republic of Ireland is a humane means of capturing badgers.

#### 1. Introduction

The badger (*Meles meles*) is recognised as the principal wildlife reservoir of *Mycobacterium bovis* infection in Ireland (Eves, 1999). Infection in the badger population contributes to the spread and persistence of tuberculosis in the cattle population (Gormley and Collins, 2000). The significant drop in prevalence of tuberculosis in cattle following the removal of infected badger populations in both the East Offaly study and Four Area study has clearly established the significance of the reservoir of infection in the badger population (Ó Máirtín *et al.*, 1998; Griffin *et al.*, 2005).

In Ireland, the badger is protected under the Wildlife Act, 1976 and the Wildlife Amendment Act, 2000, and may only be removed under license and in accordance with conditions specified by the National Parks and Wildlife Division, Department of Environment, Heritage and Local Government. As part of the strategy to control bovine tuberculosis, the Department of Agriculture and Food remove badgers where they have been identified as a probable source of an outbreak. Badgers are captured within a 2km radius of the affected farm using stopped restraints made of multi-strand steel wire wound around a core of nylon filament and designed to close to a minimum circumference of 28cm. Stopped restraints have also been used in capture-release studies (Cheeseman and Mallinson, 1979; Southey *et al.*, 2001). The method is deemed to be humane when applied in accordance with the rules outlined by the Department of Agriculture and Food (DAFF, 1996). However, to date no systematic studies have been done to assess one aspect of badger welfare, that of physical injuries, arising from this method of restraint.

The aim of this study was to determine the frequency and severity of trauma occurring when badgers were captured using stopped restraints. Trauma at the site of the restraint was assessed at post-mortem examination, and an objective visual classification schema for trauma was devised to standardise the description. Variables that may have influenced the presence and severity of traumas, for example, age, sex, weight, chest girth, restraint girth (girth of animal at level of restraint), stop distance and the location of the restraint on the body, were examined.

#### 2. Materials and Methods

Badgers (n=198) from culling operations conducted from October to December 2005 were examined. They were obtained from 17 District Veterinary Office areas covering 16 counties. Badgers were captured using stopped restraints which were 143 cm long and constructed of a multi-strand steel wire wound around a core of nylon filament. The restraints were fitted with a stop to prevent them closing beyond a minimum circumference specified by DAFF (1996) as 28 cm. However, variation in the stop distance was suspected. The restraint was secured to the ground by an angle iron and wooden support stakes. At the entrance to badger setts one or more restraints were placed in close proximity, while single restraints were laid on badger tracks. The restraints were set on 4 consecutive nights and examined each morning. Captured badgers were anaesthetised with ketamine hydrochloride (0.1 ml/kg) and medetomidine (Domitor<sup>®</sup>; 0.1 ml/kg) administered by intramuscular injection, then euthanased with an overdose of intravenous pentobarbital sodium. The badger carcases were kept at ambient temperature, and then at 4<sup>o</sup>C if not examined post mortem within 12 hours. Most post mortem examinations were conducted within 24 (range 8 - 48) hours of euthanasia.

The conduct of the post mortem examination was standardised to enable analysis of the results. The age (age classes: young <1 year, adult or old based on an assessment of tooth wear), sex, body weight of each badger was recorded. A detailed post mortem examination was conducted for the diagnosis of tuberculosis, including the collection of head and body lymph nodes and all major abdominal and thoracic visceral organs for detailed microbiological examination. The location of the restraint was recorded for each badger (Figure 1), as was chest girth, the girth at the position of the restraint and the stop distance for the restraint. A biopsy of skin, subcutaneous tissue and muscle was taken from a point 3 cm to the left of ventral midline at the level of the restraint and fixed in 10% formalin (Figure 1). The skin, subcutaneous tissues and muscle were examined visually and classified as to the degree of injury seen (Table 1). In the description of muscle injury, a class "haematoma" was included based on anecdotal reports of this occurring. Although trauma at the site of the restraint was routinely recorded, evidence of trauma at other sites was also noted, although not systematically.

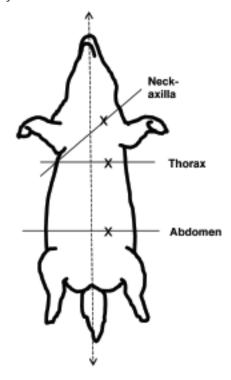


Figure 1. The locations of the restraints on the badger. The biopsy sites are indicated (X)

Table 1. Classification of injuries to badgers due to capture in stopped restraints as assessed by visual examination at post mortem

Level	Classification	Definition
Skin	No damage	No marking of skin or hair due to restraint
	Minor abrasion	Minor skin abrasions but no break in the skin
	Severe abrasion/bruising	More severe abrasion and/or bruising of the skin but no break in the skin
	Cut	Restraint has cut the skin
Subcutaneous Tissue	No damage	No oedema or haemorrhage
	Localised oedema/petechial haemorrhage	Oedema or petechial haemorrhage extending <10cm
	Moderate oedema/petechial haemorrhage	Oedema or petechial haemorrhage extending 10-20cm
	Extensive oedema/petechial haemorrhage	Oedema or haemorrhage extending >20cm
Muscle	No damage	No observable damage
	Bruising	Slight muscle haemorrhage but no tearing of muscle
	Tearing/haemorrhage	Tearing of muscle and severe haemorrhage
	Haematoma	Haematoma formation in muscle

Data on chest girth and body weight were analysed using the Student t-test; restraint girth and stop distance using the Mann-Whitney test; and location, sex and age using Fisher's exact test. The degree of association between body weight and chest girth and restraint girth, and between chest girth and restraint girth was measured using Pearson's correlation coefficient. All statistical analyses were done using SPSS version 12.0.1 for Windows (www.spss.com).

#### 3. Results

The sex and age of the 198 badgers examined in this study are shown in Table 2. The 198 badgers were captured with 211 restraints: 187 badgers were captured with one restraint, 9 with two restraints and 2 with three restraints. Data were collected separately for each restraint site, irrespective of whether there were one or more restraints at this site. In total, there were 204 separate restraint sites from 198 badgers. Restraints were positioned at the thorax, (n = 129, 63.2%) abdomen (n = 64, 31.4%) and diagonally across from the neck to the axilla (n = 11, 5.4%; Table 3). The mean weight of male badgers was 10.2 kg (sd  $\pm$  1.7) and that of females was 9.9 kg (sd  $\pm$  1.5). The mean chest girth of males was 44.1 cm (sd  $\pm$  3.9) and that of females was 44.2 cm (sd  $\pm$  3.7). The median restraint girth of males was 43.0 (range 27-52) cm and that of females was 43.0 (range 34-50) cm. The differences in weight, chest girth, and restraint girth for males and females were not statistically significantly (t-test with equal variances, *P* = 0.166 (weight), *P* =0.759 (chest girth), Mann-Whitney test *P* = 0.083 (restraint girth)). The median stop distance of the restraints was 28.5 (range 24 – 31) cm.

Table 2.	The sex a	and age of	the 198	badgers captured in stopped restraints
Sex		Age		Total
	Young	Adult	Old	
Female	11	96	8	115 (58.1%)
Male	16	66	1	83 (41.9%)
Total	27 (13.6%)	162 (81.8%)	9 (4.5%)	198

Injury	I	Location of the restra	aint	
	Thorax (%)	Abdomen (%)	Neck-Axilla (%)	Total (%)
Skin				
No damage	6 <sup>b</sup> (4.7%)	3 (4.7%)	0	9 (4.4%)
Minor abrasions	117 (90.7%)	56 (87.5%)	11 (100%)	184 (90.2%)
Severe abrasions/bruising	4 (3.1%)	3 (4.7%)	0	7 (3.4%)
Cut	2 (1.6%)	2 (3.1%)	0	4 (2.0%)
Subtotal	129	64	11	204
Subcutaneous Tissue				
No damage	4 (3.1%)	4 (6.3%)	0	8 (3.9%)
Localised oedema/				
petechial haemorrhage	95 (73.6%)	54 (84.4%)	0	149 (73%)
Moderate oedema/				
petechial haemorrhage	29 (22.5%)	6 (9.4%)	9 (81.8%)	44 (21.6%)
Extensive oedema/				
haemorrhage	1 (0.8%)	0	2 (18.2%)	3 (1.5%)
Subtotal	129	64	11	204
Muscle				
No damage	89 (69%)	60 (93.8%)	4 (36.4%)	153 (75%)
Bruising	39 (30.2%)	2 (3.1%)	7 (63.6%)	48 (23.5%)
Tearing/haemorrhage	1 (0.8%)	2 (3.1%)	0	3 (1.5%)
Haematoma	0	0	0	0
Subtotal	129	64	11	204

Table 3. Location and severity of injuries observed in 198 badgers captured in stopped restraints <sup>a</sup>

<sup>a</sup> 6 animals had restraints at two positions on the body resulting in a total of 204 restraint injury recordings

<sup>b</sup> Number of observations

#### 3.1 Skin trauma

In the majority of cases (193 badgers; 94.6%), either no skin trauma or minor abrasions were recorded. More serious classes of skin trauma were observed in 11 (5.4%) animals, including severe skin abrasion and bruising (7 badgers) and cuts in the skin (4) (Table 3). In these latter animals, the damage included single cuts less than 1 cm long (2), a 4 cm long cut (1) and seven 1 - 2 cm cuts along the line of the restraint (1).

The location of the restraint had little influence on the degree of skin trauma. No or minor skin abrasions were observed in 95.4% of cases where restraints were located on the thorax, in 92.2% of cases where restraints were located on the abdomen and in all cases where the restraint was located on the neck-axilla (Fisher's exact test P = 0.93).

When the first two classes of skin trauma (Table 3) were compared to the more serious skin trauma, the lighter animals (t-test equal variances assumed, P = 0.013), and those with smaller restraint girths (Mann-Whitney test P = 0.020) had a greater risk of more serious skin trauma.

There was no statistically significant association between skin trauma and either sex (Fisher's exact test P = 0.285), stop distance (Mann-Whitney test, P = 0.225), chest girths (t-test equal variances assumed, P = 0.063) or age (Fisher's exact test P = 0.100).

#### 3.2 Subcutaneous tissue trauma

No damage to the subcutaneous tissues was recorded in 8 cases (3.9%), localised damage in 149 cases (73.0%), moderate damage in 44 cases (21.6%) and extensive damage in 3 cases (1.5%) (Table 3). There was a statistically significant association between location of restraint and subcutaneous trauma (Fisher's Exact Test, P < 0.001), with moderate or extensive subcutaneous trauma present in 9.3% badgers captured with abdominal restraints, 23.3% with thoracic restraints and 100% with restraints located at the neck-axilla. A statistically significant association was also found between age and subcutaneous tissue trauma, when the first two classes (no damage, localised oedema/petechial haemorrhage; Table 3) are compared to the more serious trauma. As illustrated in Figure 2, 55.6% of old animals showing moderate trauma compared to 19.8% of adults and 33.3% of young animals (Fisher's Exact Test, P = 0.020). No statistically significant association was seen between damage to the subcutaneous tissues and either sex (Fisher's Exact test, P = 0.401), weight (t-test equal variances assumed, P = 0.390), chest girth (t-test equal variances assumed, P = 0.743), restraint girth (Mann-Whitney test, P = 0.177) or stop distance (Mann-Whitney test, P = 0.754).

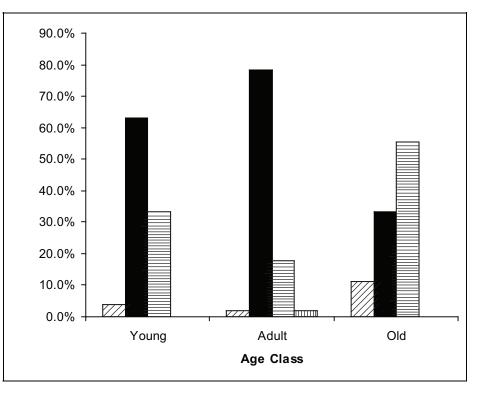


Figure 2. The breakdown of subcutaneous tissue injuries recorded in the three age groups (young, adult, and old). Diagonal = no damage, plain = localised oedema/petechial haemorrhage, horizontal = moderate oedema/petechial haemorrhage, vertical = extensive oedema/haemorrhage

#### 3.3 Muscle trauma

No muscle damage was seen in 153 cases (75%) with a further 48 cases (23.5%) and 3 (1.5%) cases having slight and more serious injury, respectively. No case of haematoma formation was recorded (Table 3).

A statistically significant association was found between restraint location and muscle damage with 63.6% of restraints located at the neck-axilla resulting in muscle damage, compared with 6.2% of restraints located at the abdomen and 31% of restraints located at the thorax causing muscle damage (Fisher's Exact Test, P<0.001). A statistically significant association was also found between age and muscle damage with 77.8% (7/9) of old animals showing muscle damage, compared to 33.3% (9/27) of young animals and 21.6% (35/162) of adults (Fisher's Exact Test, P = 0.001). Similarly there was a statistically significant association between weight and muscle damage, with lighter animals having more serious injuries (t-test equal variances assumed P = 0.045).

There were no statistically significant association between damage to muscle and either sex (Fisher's Exact test, P = 1.0), chest girth (t-test equal variances assumed, P = 0.397), restraint girth (Mann-Whitney test, P = 0.088) or stop distance (Mann-Whitney test, P = 0.542).

#### 3.4 Multiple restraints

Badgers captured with more than one restraint were more likely to sustain more serious trauma, particularly when the multiple restraints were at the same site. Of such animals, 16.7% (1/6) had a cut to the skin while 33.3% (2/6) had moderate or severe subcutaneous trauma. One badger that had been captured and escaped with the restraint still attached, was recaptured in the same area two nights later. One of the restraints lay at the thorax and the other at the abdomen. The skin trauma at both sites was minor and no damage to muscle was observed at either site. The subcutaneous damage at the thorax was moderate with the oedema/petechial haemorrhage extending down the right foreleg. At the abdomen, the subcutaneous damage was localised.

#### 3.5 Other observations

Injuries at sites other than where the restraint was located were observed in 6 (3.03%) badgers. One badger had superficial skin abrasions on its chin, lips, one hind foot and the nail bed of one fore foot. This badger had multiple 1-2 cm cuts in the skin underlying the restraint. Another badger had a broken nail on her right hind foot and the remaining four suffered only scuffed nails, minor skin abrasions or a superficial skin abrasion on a lip.

Time of euthanasia was recorded for 41 badgers, ranging from 0745h to 1215h. For these animals, the average time between daylight to euthanasia was 154 (maximum 335, minimum 30) minutes. No association was found between time and level of injury recorded at any of the three levels (t-test, equal variances assumed, skin P = 0.378, subcut P = 0.571, muscle P = 0.658).

There was a significant correlation between body weight and chest girth (r = 0.70, P < 0.001) and body weight and restraint girth (r = 0.73, P < 0.001) and between chest girth and restraint girth (r = 0.79, P < 0.001).

#### 4. Discussion

In this study, there was very limited evidence of trauma to badgers in association with the use of stopped restraints. The most severe injuries, which were regarded as clinically-significant injuries (that is, injuries that may have impaired mobility and normal behaviour), were seen in only four (2.0%) badgers. Two of these animals had extensive skin cuts and tearing and haemorrhage of muscle and the other two animals had extensive subcutaneous oedema extending down the forelimb as the restraints were located at the neck-axilla. These injuries may have temporarily impeded the badgers' ability to move around and obtain food had they been part of a capture-release programme.

A range of factors may have influenced the degree of injury sustained, including sex, body weight, age, chest girth, restraint girth, position of restraint on the body, the stop distance of the restraint and the time from daylight to euthanasia. Low body weight was associated with more serious skin and muscle injuries, but not with subcutaneous injury. Smaller restraint girths were also associated with more severe skin injuries as were smaller chest girths, although this association was not statistically significant. Body weight, chest girth and restraint girths are correlated as badgers with lower body weight have smaller chest and restraint girths. We speculate that less firm fitting restraints on lighter animals may encourage them to struggle more than heavier animals. Age was associated with injury at the subcutaneous and muscle levels, with a significantly lower percentage of older badgers having no muscle damage but a significantly higher percentage of them having extensive subcutaneous injuries than young or adult animals. The mean weight of old badgers (10.1kg) was lower than that of adults (10.4kg) but higher than that of young badgers (8kg) ruling out a weight-age interaction. The group size of old badgers was small (n = 9) and may need to be re-examined in further studies with larger numbers.

The location of the restraint on the body had a significant effect on the degree of subcutaneous and muscle injuries, with more serious trauma seen when the restraint was lying diagonally across the neck to the axilla than when located at either the thorax or abdomen. This may have been due to greater pressure on the axillary blood vessels and lymphatics, or perhaps the development and greater spread of oedema at this location, as a consequence of dependency. However, few badgers (5.6%) were caught at the neck/axilla in this study. Eleven badgers (5.6%) were captured with more than one restraint. This occurred where several restraints were laid at the entrance to a sett. Badgers captured with multiple restraints at the same location tended to have more damage than those captured

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with a single restraint, probably as a consequence of increased pressure at the site of the restraint. It is recommended that restraints are set to minimise the risk of capture with multiple restraints. The fact that a badger escaped and was recaptured in the same area two nights later suggests that this badger exhibited no strong aversion to returning to the site of his original capture.

In this study, no association was found between injury sustained and either sex, stop distance or time from daylight to euthanasia. The lack of association between sex and injury is not surprising as the weight, chest girth and restraint girth ranges for males and females was very similar. The restraint design standard specified a stop distance of 28cm (DAFF, 1996) but a range of 24-31cm was found. Although we found no association between stop distance and the degree of injury, the issue of standardisation of the product should be addressed. The exact period a badger spent in the restraint was not known but could not have exceeded eighteen hours. Time spent in the restraint may have influenced the degree of damage. While no association between the daylight hours in a restraint and the degree of injury recorded was found, it is appreciated that although this may be a period of stress for the usually nocturnal badger, this is not an entirely satisfactory measure of the time a badger had been in a restraint. In a study in the UK on the use of cage traps, they also found that the time spent in the trap from daylight to euthanasia was not associated with the degree of trap-related injuries (Woodroffe *et al.*, 2005).

The results are based on univariable analysis of the data; therefore some caution is required in their interpretation. More detailed multivariable analysis is pending. All the badgers in this study were captured over an eight-week period in the autumn and a seasonal effect on the levels of injury sustained cannot be ruled out. In general the badgers were in good body condition as would be normal for this time of the year. It may be desirable to conduct similar studies covering other seasons so that the current and additional risk factors such as weather conditions, the positioning of the restraints in the environment, the condition of the restraints, the behaviour of the badger in the restraint and the time spent in the restraint, may be investigated.

In Ireland, restraints have been used routinely by the Department of Agriculture and Food as a means of capturing badgers for culling (DAFF, 1996). Restraints have the advantages of being relatively inexpensive, lightweight, easily transported, easily positioned and do not require bait. However, the use of restraints has a poor public image, based on concerns that they may be stressful or damaging to badgers and secondly that they may capture non-target species (Bourne *et al.*, 1998). Our results compare very favourably with reports from the use of cage traps. Our study found 95% of badgers received no or minor skin trauma, 77% had no or localised subcutaneous trauma, while 98.5% received no or minor muscle trauma due to capture in stopped restraints. Woodroffe et al. (2005) looked at the injuries sustained by badgers captured in cage traps and found that 88% of badgers received no detectable injury and of those that did, 72% were described as minor. However, the studies are not directly comparable. Our study scored trauma at the site of the restraint only and used an objective visual classification schema; in contrast, the UK study looked at superficial injuries and used a modification of the trauma scale given in ISO 10990-5 (1999). This trauma scale does not reflect the subtle differences in trauma at the level of the skin, subcutaneous tissue and muscle that were observed in our study.

The welfare of badgers subjected to culling should be monitored on an ongoing basis. Minimising stress and trauma to captured badgers is important from a welfare point of view. Our study shows that the severity of injuries sustained using stopped restraints is low and restraints are a humane means of capturing badgers.

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# Description of a medium term national strategy toward eradication of tuberculosis in cattle in Ireland

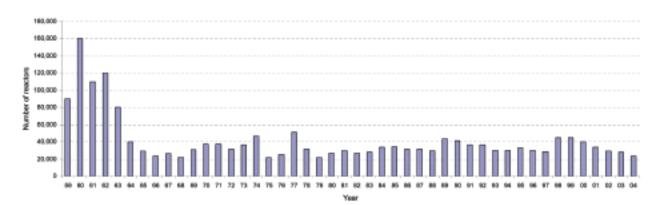
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#### Abstract

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

#### 1. Introduction

A voluntary test and slaughter scheme commenced in the Irish Republic in 1954, with an estimated 80% herds and 17% animals infected with bovine tuberculosis. The test used was the Single Intradermal Comparative Tuberculin Test (SICTT). The scheme became compulsory in 1959, involved the whole country by 1962 and has been in existence since. The Irish cattle population numbered 4.5 million animals in 250,000 herds in the late 50's, and initially test positive animals removed annually were in excess of 100,000 per annum (Watchorn, 1965). By 1965, test positive animals being removed had fallen to 40,000 animals annually, representing an animal prevalence of circa 0.5% (More and Good, 2006). At this point, progress stalled, with the testing programme consistently identifying between 20,000 and 40,000 test positives each year since 1965 (Figure 1). The national cattle herd is now 7 million bovines and these are farmed in 125,000 herds with an animal prevalence of 0.3%.





The Irish scheme conforms with animal health directive 64/432/EEC of the European Union. The SICTT skin test is applied to every herd as a screening test each year, and animals that react positively to the test are removed and slaughtered. The herd is retested at 60-day intervals from when the test positives were removed until it has passed 2 clear herd tests. In addition to this annual screening test for all herds, animals cannot be slaughtered unless they have passed a SICTT test within the previous 12 months. When infected herds are identified, neighbouring herds are tested at a frequency greater than the annual test while infection remains locally. Ancillary testing is carried out on infected herds using the interferon-Á blood test.

While such a comprehensive testing regime would be expected to successfully eradicate tuberculosis from the national cattle herd, as was the experience in many of our EU neighbours, this has not happened in Ireland nor in the United Kingdom of Great Britain and Northern Ireland. The reason for this is the presence of tuberculosis in a wildlife species, *Meles meles*, the Eurasian badger. Tuberculosis

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in this species is endemic in Ireland. Where badgers have been captured in areas where seriously infected cattle herds also exist, upwards of 40% of the badgers are culture positive for tuberculosis (L. Corner, personal communication).

The first infected badger was detected in Ireland in 1974 (Noonan *et al.*, 1975), and this led to a number of formal studies that attempted to identify a link between tuberculosis in badgers and tuberculosis in cattle in the same local areas. The first of these studied, called the East Offaly Study, was carried out between 1989 and 1994 and demonstrated that a marked reduction in levels of tuberculosis in cattle were observed when the local badger population was maintained at low levels (Dolan *et al.*, 1995). This study was confined to one geographic area, and as such was not representative of other land types found in Ireland. The results from the study, while compelling, were not conclusive findings. A follow-up study, the Four Area Project (FOP), was carried out at 4 sites between 1998 and 2002 and reductions in tuberculosis in cattle were again measured following the removal of badgers locally (Griffin *et al.*, 2005). These studies have shown that reducing the density of badgers over a wide area (the removal sites in each of the four areas averaged circa 250 km<sup>2</sup>) to perhaps 20% of their original density and maintaining these lower densities over a number of years resulted in significantly lower levels of tuberculosis in cattle locally than had been observed prior to the commencement of the trials.

While it is acknowledged that eliminating badgers in Ireland would result in a more successful cattle tuberculosis eradication scheme, such a policy would be unacceptable. The destruction of one of our important native mammal species would be completely unthinkable. The EU is a signatory of the 1989 Convention on the Conservation of European Wildlife and Natural Habitats (Berne Convention), and the Irish government ratified this treaty in 1982. Under Irish Law (The Wildlife Act, 1976), the Eurasian badger is a protected species including protection of the underground burrows (setts) where badgers live and breed their young.

#### 2. Components of the National Strategy

#### 2.1 Social partnership

In Ireland since 1987, a key component in the shaping of government policy has been a process termed "Social Partnership". Over the period since 1987, there have been 5 agreements between the Government of the day and groupings that are referred to collectively as the "social partners". The social partnership is built on 4 pillars (Trade Unions, Employers and Business representatives, Farming representatives and Community and Voluntary groupings) who with government agree a programme that incorporates a wide range of measures such as taxation matters, wage rises and elements of social/economic policy. The process has led to a prolonged period of economic stability and to levels of national prosperity that were not attainable previously. During the years 1991 to 2002, economic growth in the Irish economy averaged 7.3% per year albeit starting from a low base compared to then EU averages. These levels of growth matched the expansion in the economies of Singapore and China during the period and spawned the labels "the Celtic and Asian Tigers" in the popular press.

The current interim eradication strategy is based on agreements reached in the partnership process of 2000 (Programme for Prosperity and Fairness, 2000). The mechanism whereby the matters agreed by the government and the social partners become incorporated into policy measures, involves further negotiation between the government departments/agencies responsible for the policy areas and the individual groups from within the social partnership structure most closely involved in the area. In this case the Department of Agriculture and Food (DAF) is responsible for running the statutory bovine tuberculosis eradication programme and the Parks and Wildlife division of the Department of Environment, Heritage and Local Government are responsible for implementing the Wildlife Act, 1976, under which badgers (*M. meles*) are a protected species. The social partners with an interest in the area are the representatives of the farming bodies and the representatives of the conservation organisations such as Irish Wildlife Trust and Badgerwatch Ireland. The objective of the interim strategy is that it conforms with the spirit of what was agreed by the negotiators of the primary PPF document (Table 1).

#### Table 1. The relevant extract from the PPF section on animal disease eradication (page 68) states:

15. All parties involved agree to the adoption of necessary measures with the objective of reducing current levels of, and ultimately eradication, Brucellosis, TB and other significant animal disease from the national herd. The primary constraint on containing and eradicating TB is the existence of a significant reservoir of infection amongst the wildlife population. To reduce the incidence of TB by 50%, and to make significant progress towards the eradication of brucellosis, within the next four years, the following measures will be taken:

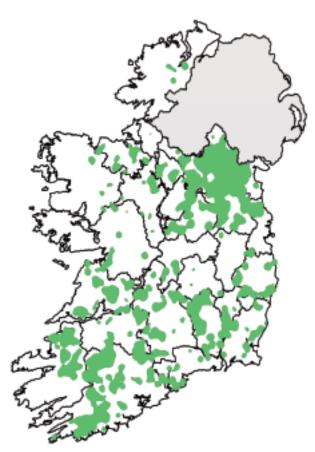
- Commit specified staff resources in each District Veterinary Office (DVO) to carry out investigative work into the cause of breakdowns; and
- In addition to current arrangements relating to wildlife, take a pro-active approach in each DVO area, using 75 dedicated Departmental and Farm Relief Service personnel, to the removal of all sources of infection in the 20% of the country which yields some 50% of the current TB reactors; the distribution of these resources will be finalised in consultation with the farm organisations.

#### 2.2 Conservation safeguards

Because the ultimate eradication of tuberculosis in cattle is contingent on reducing the levels of tuberculosis in the national badger population, a research programme is being undertaken to quantify the protective effects of vaccinating badgers with Bacillus Calmette-Guerin (BCG) vaccine. Experiments to date (L. Corner, pers. communication) have demonstrated that BCG administered orally to badgers results in protective effects following experimental infection with *M. bovis*. A large-scale field trial is planned, beginning in late 2006, where the protective effect of BCG vaccination of badgers in the wild will be evaluated. Final decisions regarding selecting components of the national strategy that will lead to the ultimate eradication of tuberculosis must await the completion of this trial.

Assuming vaccination of badgers will be part of any final strategy, and as farming must be supported under the terms of the PPF agreement, a medium term eradication strategy was formulated. Conservation of a healthy badger population nationally is a key objective of the medium term strategy, so at the heart of the strategy is a commitment on the part of DAF to guarantee that capturing of badgers will be confined, cumulatively, to not more than 30% of the agricultural land of the country over the lifetime of the strategy which began on 1<sup>st</sup>. January, 2004. At the end of 2005, capturing is ongoing on 8.8%\* of agricultural land (\*lands captured are calculated as within 0.5 km radius of any sett approved for capture).

The national capturing effort is not evenly applied, and is more intensive in those areas where tuberculosis in cattle herds is both persistent and chronic (Figure 2). Using kernelling/smoothing techniques, it is possible to delineate areas comprising roughly 30% of the agricultural land which during 1998/1999/2000 yielded roughly 70% of all standard interpretation skin test positives via the SICTT (72 hour bovine increase greater than 72 hour avian increase by more than 4mm). In the areas marked green on the map, capture will not take place over more than 60% of the agricultural land, whereas elsewhere, capturing will be capped at 20% of agricultural land. Overall, this guarantee will ensure that capturing will never exceed an area greater than 30% of the agricultural land in Ireland, or conversely badger habitats in 70% of the agricultural land will be safeguarded.



#### Figure 2. "Chronic" TB areas in Ireland

2.3 Epidemiology of tuberculosis in Ireland in 2006

Over 40 years of annual SICTT testing, involving upward of 9 million tests each year on a cattle population of 7 million along with the speedy removal of all test positives and many lower grade increases (20% of animals removed as reactors are non-standard) has altered how tuberculosis in manifested in Irish cattle herds (Martin *et al.* 2001). Clinical respiratory disease is not seen any more and carcases

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with generalised tuberculosis at slaughter are identified in fewer than 10 instances per annum from 1.5 million animals slaughtered (DAF, personal communication). Tuberculosis is now a sub-clinical disease in bovines in Ireland, and due to the annual "herd screening" testing programme infected cattle are removed and slaughtered in the early stages of the disease process. A clearer understanding of tuberculosis in Irish cattle herds is possible if one uses the concept of episodes (O'Keeffe *et al.*, 1998). An episode is the interval between a herd being placed on movement control and the lifting of movement controls. In the Irish context this spans an interval of between 150 to 200 days in the majority of herds, during which time three SICCT herd tests are applied (O'Keeffe *et al.*, 1996). Under EU rules, a herd must pass two clear herd tests at 60-day intervals following removal of test positives. A simple classification system based on episodes is outlined in Table 2 (O'Keeffe *et al.*, 1998).

	No. standard positives	No positives with gross lesions	Frequency
Group 1	2 or more	1 or more	24.5%
Group 2	1	1 or more	17.0%
Group 3	0	1 or more	2.7%
Group 4	2 or more	0	8.4%
Group 5	1	0	24.2%
Group 6	0	0	23.2%

These data represent information of 90,554 episodes, and occurred in 60,337 individual herds. In 2006, there are roughly 124,000 herds comprising cattle. Of the breakdown episodes examined during the interval, 23.2% (Group 6) did not have even a single test positive animal identified during three herd tests over a 150-200 day interval. The majority of these herds were not affected with tuberculosis, but were cases where movement controls were applied as a precautionary measure. A further 41.2% of herds (Groups 2 and 5) had only one standard test positive identified over the three tests, and while the majority of these were infected animals, the degree to which tuberculosis infection is present as a proportion of the herd is open to conjecture.

One of the elements in the PPF commitments (Table 1) was that breakdowns of tuberculosis would be investigated and a cause sought for the outbreak sought. All Group 1 and a majority of Group 4 type episodes are investigated by DAF veterinarians using a standard investigative methodology (O'Keeffe, 1999). The primary objective of this investigation is to establish if an introduced animal is the likely source of the breakdown, and if not, to establish if badgers where present in the local environment of the herd.

#### 2.4 Limited capturing of badgers

If an introduced animal(s) has been ruled out as causing the breakdown, and if signs of badger activity are found on lands of, or on lands adjacent to, the index herd, a survey of the local area is organised by DAF staff to a radius of 1 km out from the affected farm. Where setts (badger burrows) are located, the locations are recorded on a GIS database along with the sett characteristics. The majority of tuberculosis breakdowns are clustered in Ireland (O'Keeffe, 1994). Before capturing at any setts can take place, candidate setts must first be approved for capture by a member of the DAF Wildlife Unit who independently verifies that the sett is within 2 km of a tuberculosis-affected farm. Badgers are captured using a stopped-body restraint, and humanely dispatched using a 0.22 calibre bullet. Trained contractors, who are monitored and supervised by DAF staff, carry out capturing. Approval to capture a sett is contingent on the total area under capture nationally being maintained below 30% of the agricultural land as described in the previous section.

A further element of the medium term strategy is to limit any capturing during the months of January and February each year to areas that were captured previously. This measure is prompted by animal welfare concerns due to the risk of capturing lactating females that in turn would lead to the possibility of orphaned offspring. Returning to areas previously captured ensures a lower risk of capturing any badger, an even lower risk of capturing a female badger, and an even lower risk again of capturing a lactating female.

#### 3. Conclusion

The strategy outlined is a pragmatic response, based on sound science, to a complex problem. The national badger population is a valued resource and the limitations applied to the proportion of lands where capturing will be permitted guarantee the survival of the species. The objective of developing an oral delivery system of BCG that will reduce the impact of tuberculosis in badgers is realistic. Confining the capturing of badgers to areas where herds must first be identified with proven tuberculosis that was not caused by introduced infected cattle is a further safeguard against unnecessary removal of badgers. Removing heavily infected badgers from localities where cattle breakdowns have been identified will benefit the surviving test negative cattle, as well as the badgers in the wider area surrounding the removal zones. Prevaricating when confronted by a complex problem is not an option in the Irish situation. Evolving a strategy among a diverse range of stakeholders which is a sub-optimal solution for some, but which is accepted by all as a fair compromise is a triumph for common sense and a tribute to the generosity of all involved.

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## Review of Badger (Meles meles) Research Licences in 2003

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#### 1. Introduction

The National Bovine Tuberculosis Eradication Scheme includes a research programme involving diagnostic, epidemiological and wildlife investigation projects. As part of this research programme, badgers (*Meles meles*) are caught under licence and examined for evidence of tuberculosis. Data relating to licences used and badgers removed during 2003 are described in this review.

#### 2. Materials and Methods

A Veterinary Inspector, in the course of the epidemiological investigation of a tuberculosis breakdown in a herd, may make an application for a licence if wildlife involvement is suspected. Licences to remove and examine badgers are issued by the National Parks and Wildlife Service of the Department of the Environment and Local Government. Each licence is valid for one year from the date of issue and is operated by or under the direct supervision of staff of the Department of Agriculture and Food.

These local research licences are operated within a two-kilometer radius of the index farm, to assist in the epidemiological investigation of the breakdown. Other research licences, such as those granted for the Four Area Badger Study and the East Offaly Project, cover a larger area and are operated with the aim of quantifying the contribution of badgers to the spread of bovine tuberculosis.

All badgers removed under licence undergo gross *post-mortem* examination at the Regional Veterinary Laboratories of the Department of Agriculture and Food or under contract at the laboratory of the Irish Equine Centre. In addition, a number of road casualties are submitted each year, which are also examined for evidence of tuberculosis.

A proportion of badgers also had tissues submitted for further pathological tests. For example, where visible lesions of tuberculosis were found in a badger carcass this was taken as significant, and histopathological examination and culture were used as confirmatory tests in most cases. However, this was not done in all cases. Data relating to both confirmed and unconfirmed cases are presented. These extra tests were also carried out on a proportion of gross negative badgers.

#### 3. Results

#### 3.1 Origin of cases

This review presents results from 4,849 badgers submitted under local licences and the East Offaly Project. It also includes data from 439 badgers submitted as casualties. A summary of the origin of all cases is given in Table 1.

	Method of submiss	Total	
	As part of the East Offaly Project	Under local licence	
Licence	65	4,784	4,849
Road Casualty	2	437	439
Total	67	5,221	5,288

A total of 646 local licences were used in 2003. The average number of badgers removed per licence was 7.4 and the range was one to 51. Every county submitted badger carcasses under local licences in 2003.

#### 3.2 Sex Ratio

Of the 5,247 cases where sex was recorded (Table 2), the sex ratio was almost even. However, significantly more males had confirmed tuberculosis (P<0.001). The sex was not recorded in six confirmed cases. There was no significant difference in sexes with those removed under licence but significantly more males were collected as road casualties. This probably reflects the more mobile nature of males, especially during the breeding season.

Sex		Number (%) of badgers				
	Total	Badgers with	Badgers removed	Road casualties		
		confirmed TB	under licence			
Female	2,599 (49.5)	271 (40%)	2,403 (49.9%)	196 (45.8%)		
Male	2,648 (50.5)	407 (60%)	2,416 (50.1%)	232 (54.2%)		
Total	5,247	678	4,819	428		

#### 3.3 Weight

Weight was recorded in 4,175 cases and average weights are shown in Table 3. The range was 0.5 kg. to 17.0 kg for males, and 1.2 kg to 16.1 kg. for females.

23. Distribution of weight for 4,175 badgers examined during 2003					
Average weight (kg)					
	All badgers	Badgers with confirmed TB			
	9.6	9.5			
	9.1	8.9			
rs	9.3	9.2			
	Distribution o	Av All badgers 9.6 9.1			

No significant weight difference was found between badgers positive and negative for tuberculosis.

#### 3.4 Disease profile

#### a. Post-mortem examination

Almost all badgers underwent gross *post-mortem* examination. For various reasons, no results were recorded in six cases. In addition, further laboratory tests were carried out on a number of badgers. Results of the gross *post-mortem* examination of badgers are presented in Table 4. Some 6.7% of cases were found to be positive for tuberculosis and a further 21.3% were inconclusive. However, the gross *post-mortem* is not an accurate indication of tuberculosis status; for example, of the 354 badgers with visible lesions on gross *post-mortem*, only 199 of 301 cases that received further laboratory tests were confirmed (66.1%).

Table 4. Results of gross post-mortem examination							
Result	lt Removal method (number, %)						
	Under Licence	Road Casualty	Total				
Positive	323 (6.7%)	31 (7.1%)	354 (6.7%)				
Inconclusive	1,016 (21%)	108 (24.7%)	1,124 (21.3%)				
Negative	3,505 (72.3%)	299 (68.3%)	3,804 (72%)				
Total	4,844	438	5,282				

#### b. Additional laboratory testing

The additional laboratory tests consisted of histopathological examination and culture of selected lymph nodes or other tissues. The application of these tests was not uniform due to different resources at different laboratories. Thus, there is a geographical bias towards particular cases. There is also a disease bias, as the "inconclusive" cases received a disproportionate number of additional tests compared with those cases that were deemed to be negative for tuberculosis on gross *post-mortem* examination. The numbers of cases in each category, based upon the gross *post-mortem* result, that underwent additional tests, together with the results of the latter tests, are shown in Table 5.

liagnostic tests, of badgers examined during 2003					
Result		Number			
At gross	After completing				
examination	additional				
	diagnostic tests				
Positive	Positive	199			
Positive	Inconclusive	15			
Positive	Negative	87			
Positive	Not done	53			
Sub-total		354			
Inconclusive	Positive	305			
Inconclusive	Inconclusive	3			
Inconclusive	Negative	802			
Inconclusive	Not done	14			
Sub-total		1,124			
Negative	Positive	180			
Negative	Inconclusive	1			
Negative	Negative	839			
Negative	Not done	2,784			
Sub-total		3,804			
Totals		5,282			

Table 5. Results at gross examination, and after completing additional diagnostic tests, of badgers examined during 2003

Of the 354 gross positive cases, 301 (85.6%) had additional tests; 199 (66.1%) of these were confirmed. Of 1,124 gross inconclusive cases, 1,110 (98.8%) had additional tests and 305 (27.5%) of these were confirmed as positive for tuberculosis. Of 3,804 gross negative cases, 1,020 (26.8%) had additional tests and 180 (17.6%) of these were also confirmed. Overall, tuberculosis was confirmed in 684 (12.9%) badgers. A comparison of rates of confirmed infection over the past five years is given in Table 6.

Year	No. examined	Animals confi	rmed positive	
	(gross post- mortem)	(by additio	onal tests)	
		Number	%	
1998	1,997	258	12.9	)
1999	3,457	458	13.2	2
2000	4,757	580	12.2	2
2001	3,542	463	13.1	
2002	5,945	743	12.5	;
2003	5,288	684	12.9	)

Of the 439 road casualty cases, 65 (14.8%) were confirmed with tuberculosis.

#### c. Location of lesions

The locations of lesions in 351 badgers in which a single location was recorded are presented in Table 7. In a further 310 cases, lesions were recorded in more than one location; their distribution is shown in Table 8. No location was recorded for 23 cases that were originally gross negative.

Table 7. Location of lesion in 351 confirmed ca	ses with	Table 8. Location of lesions in 310 cases w	th multiple			
a single lesion		lesions				
Lesion location		Lesion location				
Axillary lymph node	5	Axillary lymph node	30			
Bronchial/Mediastinal lymph nodes	62	Bronchial/Mediastinal lymph nodes	171			
Hepatic lymph node	8	Hepatic lymph node	38			
Kidney	14	Kidney	34			
Liver	8	Inguinal	0			
Lung	41	Liver	29			
Mesenteric lymph node	9	Lung	127			
Pharyngeal, parotid or submandibular lymph node	97	Mesenteric lymph nodes	29			
Popliteal lymph node	57	Pharyngeal, parotid or submandibular lymph node	167			
Prescapular lymph node	36	Popliteal lymph node	70			
Skin	8	Prescapular lymph node	105			
Other location <sup>a</sup>	6	Skin	19			
		Spleen	10			
Total	351	Other location <sup>a</sup>	11			
<sup>a</sup> Other locations including inguinal gland, pericard	lium,	<sup>a</sup> Other locations included pleura, heart, inguinal g	land, adrena			

tesions	
Lesion location	
Axillary lymph node	30
Bronchial/Mediastinal lymph nodes	171
Hepatic lymph node	38
Kidney	34
Inguinal	0
Liver	29
Lung	127
Mesenteric lymph nodes	29
Pharyngeal, parotid or submandibular lymph node	167
Popliteal lymph node	70
Prescapular lymph node	105
Skin	19
Spleen	10
Other location <sup>a</sup>	11
<sup>a</sup> Other locations included pleura, heart, inguinal gl. gland and tonsils	and, adrenal

#### 3.5 Licence results

pleura and omentum

Overall, of the 646 licences used, 293 (45.4%) yielded confirmed tuberculous badgers. Again it should be stated that many badgers that had no gross evidence of tuberculosis had not been subjected to any further confirmatory tests.

As the number of badgers removed under a licence increased, the greater the likelihood of finding one with tuberculosis. Density of setts in areas will vary depending on habitat type (Smal, 1995) and this is reflected in the number of badgers removed under individual licences. Interference at setts by blocking, digging etc. continues to be reported. This also results in lower numbers being captured at these setts. In other situations, the number of positive cases may have been reduced because of the prior death of affected badgers.

#### 4. Discussion

These data demonstrate that the badger population examined here had a minimum rate of infection of 12.9% in 2003 (Table 6). This is remarkably similar to the rate of infection in badgers disclosed in previous years. In the final year of the Four Area Badger Study, from September 2001 to August 2002, some 290 badgers were examined of which 35 (12.1%) were confirmed with tuberculosis (Griffin et al., 2003). In an analysis of cattle herd breakdowns, a 60-96% decrease was found in the rate at which herds were becoming the subject of a confirmed restriction in the badger removal area compared to the reference area (Griffin et al., 2005).

The population studied was biased towards infected groups, as the initial selection was in areas of cattle herd breakdowns. Nevertheless, the presence of tuberculosis in 14.8% of the 439 road casualties examined, i.e. a more representative sample, and the fact that positive badgers were found in every county in 2003, supports the view that tuberculosis is endemic in the Irish badger population.

The fact that significantly more males than females were found to be tuberculous is attributed to the extra contact and fighting which occurs in the breeding season. Similar findings were reported in badgers in 1997 (O'Boyle, 1997), 1999 (O'Boyle 1999) and 2000/2001 (O'Boyle 2001), 2002 (O'Boyle et al., 2003) but not in 1998 (O'Boyle 1998).

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

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## Abstract

Tuberculosis is a significant health problem of captive deer in a number of countries. This paper describes outbreaks of bovine tuberculosis in two Sika deer farms in southeast Ireland, and the methods used to control the disease. On Farm A, infection in Herd A was first detected during 1994. A second outbreak, with a different strain of *Mycobacterium bovis* possibly from a local badger, was detected in 2002. Infection was particularly prevalent in two groups of young deer. Control (but not eradication) has been achieved using testing and removal in association with segregation of young animals. In Herd B, located on Farm 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 a more realistic option in the short- to medium-term.

## 1. Introduction

Tuberculosis (TB) in deer has been diagnosed in every country where deer are managed as a domestic species (Griffin and Buchan, 1994). A range of strategies has been developed by national agencies to deal with this problem, generally in line with existing national control programmes for bovine tuberculosis. Different methods have been used to manage and identify TB in deer, varying from effective immunodiagnostic tests and selective breeding to novel microbiological and immunological concepts (Griffin and Mackintosh, 2000). There may also be a component of genetic resistance to tuberculosis (Adams and Templeton, 1998). DNA fingerprinting has been used to determine the source of disease, and DNA vaccines are being appraised as a means of controlling disease and differentiating vaccinated from infected animals.

In Ireland, there are relatively few farmed deer. Approximately 500 farmed herds were present in Ireland in 1995, but 200-250 farms (averaging 50-60 deer per herd) remain today, reflecting problems with the economic viability of farmed deer enterprises. Most Irish venison is exported.

The prevalence of TB in the Irish wild deer population is currently unknown, although a prevalence of 4% was recorded in a limited survey in the early 1980s (Dodd, 1984). Although the prevalence of TB in farmed deer is unknown, our experience is that the disease is a major problem in some herds. TB in deer is a notifiable disease in Ireland under the Diseases of Animals Order (1992). In addition, trade in live deer within the European Community is subject to certification of freedom from tuberculosis and brucellosis under the European Communities Trade in Animals and Animal Semen, Ova and Embryos Regulations (1996). Although there is no legal obligation for Irish deer farmers to test their herds for TB, deer are subjected to a post mortem examination at slaughter.

This paper describes outbreaks of bovine tuberculosis in two deer farms in southeast Ireland, and the methods used to control the disease.

## 2. Materials and methods

#### 2.1 The case farms

The two case farms, each farming Sika deer, are located in southeast Ireland. Farm A consists of 75 acres, of which 70 acres are fenced for the purposes of deer farming. The 70 acres is subdivided into 3 sections by internal fences. Similarly, 30 of 40 acres of Farm B are fenced, and subdivided into 6 sections. The perimeter and internal fencing on both farms are made of wire mesh, which is 1.8 meters high and staked at intervals. Each farm has a raceway, to facilitate the flow of deer into the crush, and a pen area, which is used for dividing, separating and handling individual deer. The pens are interconnected and dividing doors are easily opened and closed to increase and decrease space as necessary. Individual pens are able to hold up to 20 deer. The crushes on both farms are adjustable, to handle deer of different sizes. Ten adult deer to the acre represent the optimum stocking density. Calves are weaned normally during November and are then moved to a new location. Apple pulp is the main supplementary winter feed, complemented with hay or dried beet nuts. The deer on both farms are out wintered and there is adequate cover and shelter for the animals in adverse weather conditions. Apple pulp is fed on a daily basis, usually in the morning and is deposited on the ground. Neighbouring farms are mainly beef and dairy establishments and there is one sheep flock. TB breakdowns have occurred in these cattle herds in the past.

## 2.2. Diagnostic methods

#### 2.2.1 Single Intradermal Comparative Cervical Test (SICCT)

On each farm, tuberculin testing was conducted using the single intradermal comparative cervical test (SICCT). On the two case farms, good handling facilities were available, enabling these tests to be conducted with precision. As deer were mostly tested indoors, artificial lighting was used on dull days. Sika deer, due to their smaller size and quieter temperament, are much easier to handle then either fallow or red deer. Experienced personnel were present on each farm during the testing procedure. Care was taken to ensure that deer were handled safely and securely at all times, thereby avoiding injury to handlers or animals. The same tester initiated and completed each SICCT, as techniques may differ between individuals with measurements varying accordingly.

The SICCT was conducted using the following methodology. A coarse electric shears (Liscop Super 3000-Type 1300-2-TD) was used to remove heavy hair from the side of the neck, then a fine electric shears (Oyster no. 80, size 40) was used to prepare both injection sites. Each injection site was approximately 45 mm square (roughly the width of the blade), and both sites were located about 50-60 mm apart in the middle third on one side of the neck. Each site was palpated, and unusual features (lumps etc) noted. A fold of skin was lifted at each site, between the thumb and forefinger, and measured with a digital calipers. Both measurements were recorded. The upper site was injected intradermally (I/D), using a 1 cc insulin syringe and 26 gauge needle, with 0.1 cc of avian tuberculin PPD (25,000 IU/ml, Institute for Animal Science and Health, Lelystad, The Netherlands). The lower site was injected with 0.1 cc bovine tuberculin PPD (30,000 Ph. Eur. U/ml, Institute for Animal Science and Health, Lelystad, The Netherlands). Each injection was made at the centre of the relevant site. The syringes were individually loaded for each injection. A slight resistance was noted when the intradermal injection was properly executed (a lack of resistance may indicate that the injection is subcutaneous). A 'bleb', which tended to spread, was observable when the injection was properly administered, in contrast to the pea which is palpable in cattle following injection. Needles were disinfected before each injection using methylated spirits and cotton wool, and the test was conducted under strict hygienic conditions. Reactions were examined after 72 hours and measured to the nearest 0.1 mm. Precise measurements were required as the changes in skin thickness were often slight, particularly where the reaction was a diffuse oedematous plaque. The site was observed and palpated before measuring. Even small reactions were observable on occasions, and a slight reddening of the area was sometimes evident. A reaction was represented as the difference between the initial and final skin measurements at 72 hours. Severe interpretation was applied when it was established that TB infection already existed in a herd; otherwise, standard interpretation was applied (Figure 1).

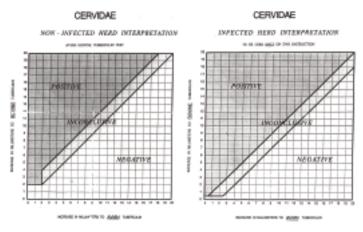


Figure 1. Standard (left) and severe (right) interpretation charts for SICCT in deer

#### 2.2.2 Additional diagnostic measures

A detailed gross post-mortem inspection was conducted in the abattoir by a veterinary surgeon of all deer at routine slaughter, and suspicious lesions were dispatched to the Central Veterinary Laboratory in Abbotstown for cultural examination. Throughout the testing programme in herd A, test-positive animals were euthanased and sent to the Kilkenny Regional Veterinary Laboratory for detailed examination.

## 2.3 Control measures

In both Herds A and B, regular skin testing was conducted and all positives under severe interpretation were removed. Clinical suspects (*e.g.* animals with unexplained weight loss) were also euthanased and examined in the Regional Laboratory. In addition, following the spring and autumn tests in 1994, young clear stock in Herd A were separated from the older males and females, and reared as a separate herd in an isolated section of the farm. These animals never again came in contact with the older stock.

#### 3. Results

## 3.1 Deer, prior to 2000

Infection with bovine tuberculosis was first detected in Herd A during routine post-mortem examination of deer slaughtered in an abattoir in 1993. In herd B, infection was first detected following an onfarm death in 1995.

TB testing commenced in herd A during spring 1994. Herd B, which originated from Herd A following the purchase of 43 breeding females and 4 stags in1991, was incorporated into a testing schedule from spring 1996. Both herds were tested twice annually where possible while infection was detectable, and annually when clear. The testing history of Herds A and B during 1994 to 2000 is presented in Table 1.

During this period (1994-2000), skin test reactors in Herd A were detected from 1994 to 1997; no visible lesions were found in the two reactors in spring 1997. During 1998, only a partial herd test was conducted. However, of 87 animals routinely slaughtered in October 1998, three had visible lesions in the mesenteric lymph nodes. These lesions had histological evidence of tuberculosis, but were negative on culture. A female culled in early 1999 because of poor condition showed gross lesions in the lung and *M. bovis* was isolated on culture. No reactors were found in two TB tests (the herd in two parts) during 2000.

Year	Season	He	rd A	Herd B				
		No. Tested	No. Positive	No. Tested	No. Positive			
1994	Spring	150	33					
	Autumn	206	38					
1995	Spring	318	74					
	Autumn	426	5					
1996	Spring	318	5	178	18			
	Autumn	305	3	115	7			
1997	Spring	363	2	110	0			
	Autumn	387	0	No test	-			
1998	Spring	82	0	137	0			
	Autumn	No test	-	No test	-			
1999	Spring	452	0	206	0			
	Autumn	82	0	No test				
2000	Spring	251	0	No test				
	Autumn	104	0	No test				

#### 3.2 Deer, during 2001

Testing was temporarily postponed in both herds during the FMD crisis in 2001.

#### 3.3 Deer, during 2002 and early 2003

In Feb 2002, a sika deer from Herd A, showing clinical signs of pneumonia, was presented for examination. TB was suspected and the deer was humanely killed. A badger with a suppurating neck wound was found dead on this farm on the same day (see Figure 2). Gross lesions of tuberculosis were detected at Kilkenny Regional Veterinary Laboratory in both animals, and *M. bovis* was subsequently recovered at the Central Veterinary Laboratory in Abbotstown (see 3.7 for details of strains).

The entire herd was then tested during March and April 2002, and results indicated severe TB infection on farm A (Table 2). At the time of testing, the herd was divided into three main groups, which were located on different sections of the farm (Figure 2). Group 3 (pond) had no physical contact with either group 1 (hill) or 2 (front field), whereas Groups 1 and 2 were separated by a wire mesh fence. The Table 2: TB testing results from Herd A during 2002 and early 2003

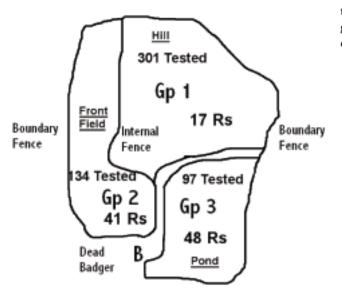
Group <sup>a</sup>	Date of testing	No. Tested	No. (%) Reactors	Gross post-mortem <sup>b</sup>	Histology
2	05/03/2002	134	41 (30.6)	21 VL	20 positive
				8 I/C	8 negative
				12 NVL	-
3	05/04/2002	97	48 (49.5)	24 VL	Not done
,	03/04/2002	51	40 (49.5)	24 NVL	NUL UUILE
1	10/04/2002	301	17 (5.6)	15 VL	Not done
	10/04/2002	501	17 (5.0)	2 NVL	NUL UUILE
2	10/05/2002	93	11 (11.8)	6 VL	Not done
-	10/03/2002	33	11 (11.0)	5 NVL	NUL UUILE
3	23/09/2002	49	11 (22.4)	6 VL	Not done
<b>)</b>	25/05/2002	49	11 (22.4)	5 NVL	NUL UUILE
1	23/11/2002	142	5 (3.5)	4 VL	Not done
	28/01/2003 <sup>C</sup>	185	2 (1.1)	1 NVL	Not done
2	04/02/2003	82	2 (2.4)	1 VL	Not done

<sup>a</sup> Group 1 consisted of adults and calves, Group 2 calves and yearlings, and Group 3 adults and yearlings <sup>b</sup> VL: visible lesions; NVL: no visible lesions; I/C: inconclusive results

<sup>c</sup> The younger animals in group 1 that had not previously been tested on 23/11/2002

infected badger was found in close proximity to Groups 2 and 3 (Figure 2)

A few weeks prior to 05/03/02, 60 calves born in June 2001 to Group 3 females were moved to the front field (Group 2), joining 74 yearlings already present in this field. At the TB test on 05/03/2002, 21 (of 60; 35.0%) calves and 20 (of 74; 27.0%) yearlings were positive. A month later, during a TB test of animals in field 3 on 05/04/2002, 34 (of 70; 48.6%) older females, 12 (of 22; 54.5%) yearlings



and 2 (of 5; 40.0%) stags were positive. Following the test on group 3 on 23/9/2002, the remainder of this group were slaughtered at an abattoir and the follow up examination failed to detect any evidence of infection.

Figure 2. Map of Farm A, highlighting the location of deer groupings and the site where a tuberculous badger was found during March and April 2002

Table 3: TB testing results from Herds A and B during 2003 to 2006													
Year	Season		Herd A		Herd B								
		No. Tested	No. Positive	No. Lesions	No. Tested	No. Positive							
2003	Autumn	343	1	1	190	0							
2004	Spring	389	0		No test								
2004	Autumn	275	0		No test								
2005	Spring	277	2	2	201	0							
2006	Spring	336	1	1	No test								

3.4 Deer, from mid 2003

The results of TB testing from mid 2003 to 2006 are presented in Table 3.

## 3.5 Badgers, from 1996 and 2002/03

Table 4: Post	le 4: Post mortem results of badgers caught around Farm A during TB breakdowns in 1996 and 2002/03												
Year	No badgers caught	No. with gross TB lesions	No. with histological evidence of TB										
1996	12	5	5										
2002/2003	43	16	14										

Data on badgers captured on and around farm A during 1996 and 2002/03 are presented in Table 4. Badgers regularly trespassed onto Farm A, as was evidenced by breaches in the surrounding fence. Badger hairs were found at these locations.

3.7 M. bovis strain types isolated during breakdowns on Farm A in 1994 and 2002

The strain type of *M. bovis* that was isolated from deer during the breakdown in 1994 was different to that which occurred in the 2002 Table 5. Strains of *Mycobacterium bovis* identified in deer and a badger during two TB breakdowns on Farm A in 1994 and 2002

Breakdown	F	RFLP analysis <sup>a</sup>		Species	
	IS6110	PGRS	DR		
During 1994					
	A1	D5	А	Deer	
	A1	D5	А	Deer	
	A1	D5	А	Deer	
During 2002					
	A1	D8	А	Deer	
	A1	D8	А	Deer	
	A1	D8	А	Deer	
	A1	D8	А	Deer	
	A1	D8	А	Badger	

<sup>a</sup> Restriction fragment length polymorphism (RFLP) analysis based on DNA probes from *IS6110*, the polymorphic GC-rich sequence (PGRS) sequence and the direct repeat (DR) sequence

breakdown. Strain types were not isolated from badgers caught during the first breakdown. However, the strain type taken from the badger in the 2002 breakdown matched those isolated at that time from the deer.

## 4. Discussion

Tuberculosis is recognised as a very important health problem of captive deer in many countries, including Japan (Yoshikawa *et al.*, 1994), New Zealand (Griffin *et al.*, 1998) and the United Kingdom (Fleetwood *et al.*, 1988). White-tailed deer are also an important

wildlife reservoir of bovine tuberculosis in the USA (O'Brien *et al.*, 2006). Several detailed reviews are available (Clifton-Hadley and Wilesmith, 1991; Griffin and Mackintosh, 2000). This study highlights the significant problems of bovine tuberculosis over an extended period on two deer farms in Ireland.

In this study, tuberculosis was rapidly eradicated from one of the case farms, but not the other. In herd B, following the initial detection of 25 SICCT reactors during 1996, subsequent skin testing combined with gross post-mortem examination at routine slaughter failed to detect any additional infected animals. This herd has tested clear at all subsequent tests; most recently in spring 2005. In contrast, effective disease control in herd A has proved much more problematic. Based on extensive studies in New Zealand, most TB breakdowns in deer herds are controlled and cleared within 18 months (Griffin and Buchan, 1994). Eradication efforts are much less effective in herds with well-established infection (more than 30% of animals infected at the index test), particularly among younger animals (Griffin *et al.*, 1998). Although the overall prevalence of infection in Herd A during 2002 was less than 30%, prevalence was substantially greater in younger than older animals (Group 2: 30.6% positive on 05/03/02; Group 3: 49.5% positive on 05/04/02). Therefore, the number of young stock infected in the 2002 breakdown was sizeable. From a total of 106 reactors from the March and April 02 tests, 68 were less than two year old.

We used the Single Intradermal Cervical Comparative Test as the primary method to detect infected animals on both case farms. Further, disease control was mainly undertaken (and eradication of infection effectively achieved in one of the case herds) through the detection and removal of reactor animals. In our experience, the test proved very useful, as highlighted by the high level of correlation between the results of the SICCT and post mortem findings. Claims for the sensitivity of the SICCT in deer vary from 80% (Stuart et al., 1988) to 84% (Kollias et al., 1982), with high levels of sensitivity being achieved in experimentally infected deer under controlled conditions. The SICCT in deer is not without practical and technical difficulties. For practical reasons, herd tests cannot be carried out during the calving (mid May to early July) and rutting (September and October) seasons. Consequently, on the case farms, testing is limited to the period between October and May. As a result, there was often a prolonged testing interval, which may have played a role in ongoing transmission of infection in Herd A. Following the skin test there is desensitization to subsequent SICCT tests, which is still detectable 60 days following the previous test. Following a test interval of greater then 60 days, the sensitivity of the SICCT was 91.4% (Corrin et al., 1993.). In this study, testing intervals were always more then 60 days and generally closer to 90 days; in some cases the intervals were even longer. Finally, in sika deer double skin thickness varies between 1.3 mm (in young deer) and 11 mm (in the case of stags). Therefore, considerable care was taken throughout the testing period, particularly in thin-skinned animals, to ensure that tuberculin was injected intradermally, and not subcutaneously. In our experience, the testing procedure is substantially more rigorous in deer then cattle, demanding significantly more input from the tester and handlers alike. Therefore, tuberculin testing is more expensive in deer than cattle, due to the slow rate of testing and lower relative cost of the animal, particularly for fallow and Sika deer. This has implications from the point of view of the industry for the economics of TB eradication in deer.

Throughout this outbreak, tuberculosis was generally confirmed by culture. During 1998, however, although tuberculous lesions were identified in 3 of 87 animals routinely slaughtered from Farm A during 1998, all were negative on culture. It is possible that all were caused by *M. avium* rather than by *M. bovis*. Unfortunately *M. bovis*, *M. avium* complex and *M. paratuberculosis* all show similar histology, which prevent differentiation on the basis of histological findings alone (Campbell, 1995). The gross and histological appearance of the lesions in the deer infected with *M. avium* were indistinguishable from those caused by *M. bovis* (de Lisle *et al.*, 1995). In a study of slaughtered farmed deer in Ireland, *M. avium* was isolated from 43 per cent of tuberculosis lesions, predominantly mesenteric and retropharyngeal lymph nodes (Quigley *et al.*, 1997).

It is likely that there were two distinct outbreaks of tuberculosis on Farm A during 1994 to the present, the first from 1994 and the second from 2002. Although it is feasible that there was carry-over of unresolved (residual) infection between 1997 and 2002, a different strain type of M. bovis was isolated in 2002 (Table 5), suggesting a new and unrelated source of infection. Further, given the potential for high levels of within-herd transmission within deer herds, infected deer must have remained non-infectious throughout this period. Based on work by Lugton et al., (1998), within-herd transmission is generally facilitated by a small number of severely-infected deer which excrete large numbers of bacilli. With the majority of infected animals, there is a low recovery of *M. bovis* from nasal, pharyngeal and tracheal swabs and faeces, suggesting that bacillary excretion from infected deer is uncommon (Lugton et al., 1998). The source of the initial outbreak (in 1994) is unknown. Although there is a history of periodic TB breakdowns among neighbouring cattle, there is little likelihood of deer to cattle contact taking place along the boundary fence. Deer escapes occur occasionally, with escaped (subsequently, feral) animals then having the opportunity of contact with cattle. In the latter outbreak, however, the same strain of M. bovis was found in deer and a badger. Although this does not prove that transmission has occurred from the badger to deer, recent work has highlighted the importance of badgers in the epidemiology of tuberculosis in Irish cattle herds (Griffin et al., 2005). In this breakdown, winter feeding may have played a role in the source - and subsequent spread - of infection. Apple pulp was fed on the ground on a daily basis, which may have facilitated contact between badger and deer over a sustained period. Badgers removed under license from the area had a high incidence of TB infection during both breakdowns. In New Zealand, it is hypothesised that the principal mode of transmission from dying tuberculous possum to deer is via the oral route or droplet inhalation, with the more dominant and inquisitive deer being the first to become infected (Lugton et al., 1997). It is recognised that tuberculous badgers with infected bite wounds die more rapidly than those with infection of the respiratory system (mean survival times 117 days and 491 days, respectively; Clifton Hadley et al., 1993). Based on experimental studies (Palmer et al., 2004), close contact between deer is known to facilitate within-herd transmission. Sika deer are social animals and tend to lie together while resting or sheltering during poor weather.

It is likely that infected badgers continue to be present in and around farm A. Therefore, re-infection from this source remains an ongoing risk. Under the circumstances, testing alone may not be sufficient to achieve and maintain TB-freedom in this herd, and control rather than eradication of infection may be a more realistic option in the short- to medium-term.

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# Survival and dispersal of a defined cohort of Irish cattle

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#### Abstract

An understanding of livestock movements 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 County Kerry during 2000. The cohort was observed for a maximum of four years, from 01 January 2000 to 31 December 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 4-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.

## 1. Introduction

TThe movement of animals is often implicated in the spread of disease; for example, foot and mouth disease (Anderson, 2002; Carrique-Mas *et al.*, 2005) and scrapie (Gubbins, 2005) in Britain, and Johne's disease in the Netherlands (Weber *et al.*, 2004). Logically, effective disease prevention, control and prediction depend in part on a sound understanding of movements in relevant animal populations. For a range of diseases, studies have been conducted to define the importance of animal movement and the potential of movement in disease transmission (Forde *et al.*, 1998; Rojas *et al.*, 2002; Sanson, 1994; Sanson *et al.*, 1993; Velthuis, 2004; Webb and Sauter-Louis, 2002). Modelling studies have also been conducted to quantify the role of animal movement in disease spread (Bachmann *et al.*, 2005; Chowell *et al.*, 2005; Gubbins, 2005; Kitching *et al.*, 2005; Mangen *et al.*, 2002; Mourits *et al.*, 2002; Nielen *et al.*, 1996; Sanson *et al.*, 1993). Such is the importance of disease transmission due to animal movements, new methodologies have been adapted from other areas of science, such as network analysis, in a further attempt to describe and predict disease spread (Bigras-Poulin *et al.*, 2004; Christley *et al.*, 2005; Webb and Sauter-Louis, 2002).

To date, no studies have been conducted to quantify the dispersal, movement and survival of Irish cattle. As a result, there is no knowledge on the potential for disease transmission as a result of these movements. The objectives of this study were to describe the movement of cattle born in county Kerry in 2000 in terms of dispersal, distance travelled and frequency of moves, as well as the survival of this cohort over a 4 year period.

#### 2. Materials and Methods

#### 2.1 The data

In Ireland, a central database is used to record the origin, identity and life history of cattle prior to death or slaughter. The database manages calf birth registrations and the Cattle Movement Monitoring System (CMMS). All cattle are uniquely identified, and farmers are obliged to maintain an on-farm Herd Register, which provides a record of all cattle in the holding, and to register the full details of births, (incoming and outgoing) movements and on farm deaths. Animal movement data are also captured electronically at livestock marts, meat plants and export points. Components of the database have been operating since the 1950s, with the system being fully-operational since 01 January 2000 (Anon., 2003).

The central database was accessed to identify all registered animals born on farms in county Kerry during 2000 and to access relevant data including animal identification, date of birth, sex, breed of sire, breed of dam and identification of the birth herd. In addition, we extracted data on all recorded movements prior to 01 January 2004, including date and type of movement, identification of the premises (and county) of origin and destination, and – if relevant – date of death on-farm. Animals were considered to be of dairy breed if both parents were Friesians, and beef otherwise.

## 2.2 Data analyses

The data were managed using Microsoft Access (Microsoft Corporation, Seattle, WA, USA). Graphs were produced using Microsoft Excel. To create a spatial representation of dispersal, files were first prepared of each relevant livestock movement; including animal identification, the herd (and county) of origin, and the herd (and county) of destination. The Microsoft Access file was then converted to text format using a programme written in Microsoft Visual Basic, stored in ArcInfo (ESRI GIS and Mapping Software, Redlands, CA, USA) and graphed using ArcView.

The cumulative probability of animals surviving to defined ages was determined using Kaplan-Meier survival curves, based on an analysis of time from birth to death. Data were right-censored if animals were either exported from Ireland on or prior to 31 December 2003, or were still alive on Irish farms on 01 January 2004. The survival curves were produced using Stata version 8 (StataCorp, College Station, TX, USA) following data transfer from Microsoft Access using Stat/Transfer (Circle Systems, Seattle, WA, USA). Survival curves were created for the birth cohort, in total and by breed (dairy versus beef) and sex.

## 3. Results

## 3.1 Survival

A total of 145,211 cattle were born in Co. Kerry during 2000, including 40,068 (27.6%; 19,650 female and 20,418 male) dairy animals (Figure 1) and 105,143 (72.4%; 51,223 female, 53,920 male) beef animals (Figure 2). The beef animals made 138,186 (average 1.3, median 1, range 0-12) moves from farm to farm or via markets during the 4-year study period. The dairy animals moved on 33,176 occasions during this period (average of 0.8 moves per animal, median 1, range 0-7).

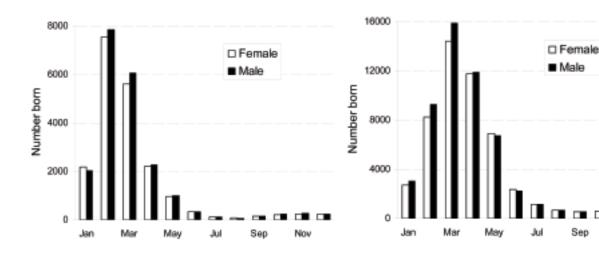


Figure 1. The number of dairy cattle born, by month and gender

Figure 2. The number of beef cattle born, by month and gender

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During the study period, 19,815 (18.8% of the 105,143) beef animals were alive on Irish farms at the end of the study, including 7,000 (6.7%) animals that had never moved from their premises of birth. Of these latter animals, 5,845 (83.5%) were female. A further 64,155 (61.0%) beef animals were slaughtered prior to study end, 3,696 (3.5%) died on-farm and 17,477 (16.7%) were exported. During the study period, 14,068 (35.1%) of the dairy cohort were slaughtered, 1,577 (3.2%) died on farm and 4,963 (13.2%) were exported. A total of 19,460 (48.6%) of the diary animals survived on Irish farms until the end of the study, including 11,235 (27.7%) of all dairy animals which never moved from their premises of birth. A total of 10,755 (95.7% of these latter) animals were female.

The cumulative probability of survival is presented in Table 1, and Figures 3-7.

**T** 1

	Survival probability														
Survival to:		Production t	уре	Se	ex	Be	ef	Dairy							
	Total	Beef	Dairy	Male	Female	Male	Female	Male	Female						
1 year	0.97	0.96	0.98	0.97	0.96	0.97	0.95	0.98	0.97						
	(0.96-0.97)	(0.96-0.96)	(0.97-0.98)	(0.97-0.97)	(0.96-0.96)	(0.97-0.97)	(0.95-0.95)	(0.98-0.98)	(0.97-0.98)						
2 years	0.77	0.72	0.89	0.81	0.73	0.80	0.62	0.83	0.94						
	(0.77-0.78)	(0.72-0.73)	(0.89-0.90)	(0.81-0.81)	(0.73-0.73)	(0.80-0.80)	(0.62-0.63)	(0.83-0.84)	(0.94-0.94)						
3 years	0.36	0.26	0.60	0.17	0.57	0.13	0.43	0.3	0.85						
	(0.36-0.36)	(0.26-0.26)	(0.60-0.61)	(0.17-0.18)	(0.57-0.58)	(0.13-0.14)	(0.42-0.43)	(0.29-0.31)	(0.85-0.86)						
4 years	0.29	0.20	0.52	0.11	0.50	0.07	0.38	0.25	0.72						
	(0.29-0.30)	(0.20-0.21)	(0.51-0.52)	(0.11-0.12)	(0.49-0.50)	(0.07-0.08)	(0.37-0.38)	(0.24-0.26)	(0.72-0.74)						

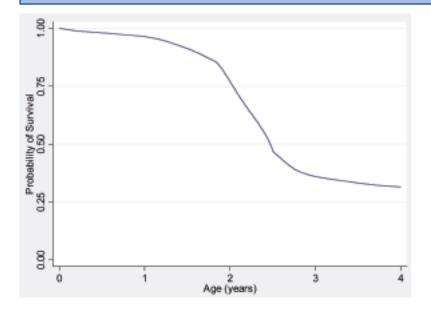
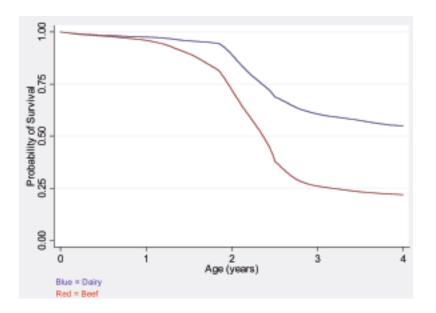


Figure 3. The cumulative probability of survival of cattle born in Co. Kerry during 2000





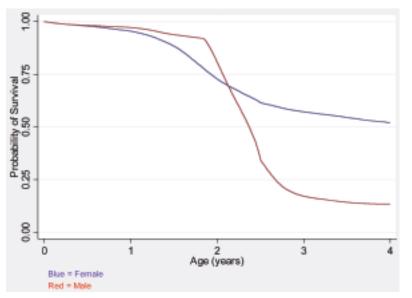


Figure 5. The cumulative probability of survival of cattle born in Co. Kerry during 2000, by sex

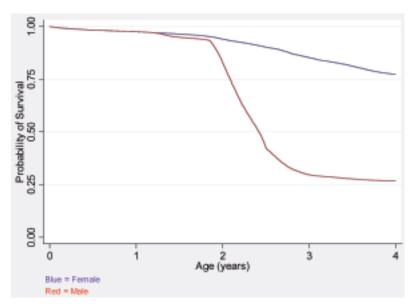


Figure 6. The cumulative survival probability of dairy cattle born in Co. Kerry during 2000, by sex

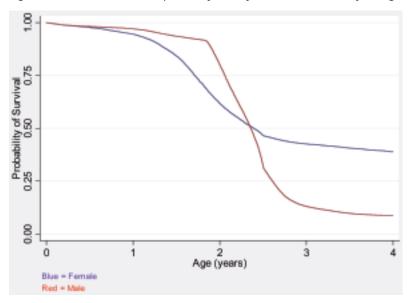


Figure 7. The cumulative survival probability of beef cattle born in Co. Kerry during 2000, by sex

## 3.2 Dispersal

The dispersal of animals during the study period is presented in Figures 8-11. Beef cattle moved an average of 49.4 kilometres per farmto-farm move (median 20 km, min <1 km, max 321 km). Dairy cattle moved an average of 44.6 kilometres per farm-to-farm move (median 19 km, min <1 km, max 326 km).

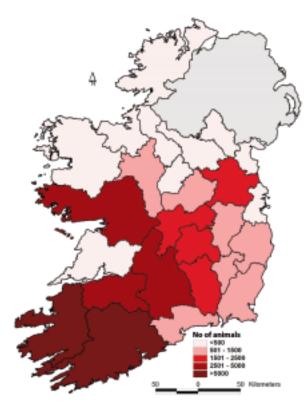


Figure 8. The location, on 01 January 2002, of beef cattle born in Co. Kerry during 2000

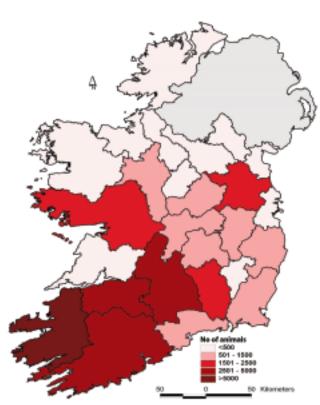


Figure 10. The location, on 01 January 2002, of dairy cattle born in Co. Kerry during 2000

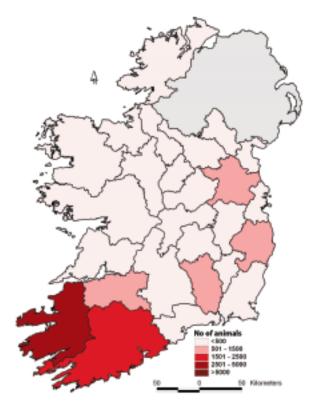


Figure 9. The location, on 01 January 2004, of beef cattle born in Co. Kerry during 2000

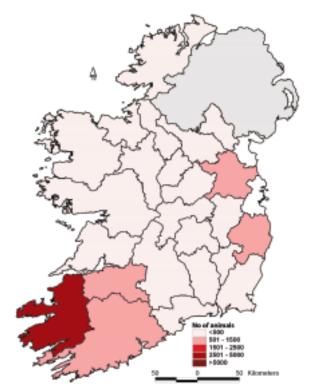


Figure 11. The location, on 01 January 2004, of dairy cattle born in Co. Kerry during 2000

# 4. Discussion

#### 4.1 Dispersal

There was substantial dispersal of cattle throughout Ireland, with dairy and beef animals from this Kerry birth cohort moving to every other county by the beginning of January 2002 (see Figures 8 and 10). As expected, dispersal was affected by distance, with counties closer to Kerry received more animals than counties that were more-distant. For example, on 01 January 2002, there were 4,706 beef animals from the birth cohort in Limerick and 6,371 in Cork, in comparison to 72 and 7 beef animals in the more-distant counties of Monaghan and Cavan, respectively. Although no account is taken of county size (or cattle population), the general trend is clear. However, the dispersal of animals is affected by more than distance alone. To illustrate, fewer animals moved to County Clare (immediately north of Kerry across the River Shannon) than might be expected. On 01 January 2002, there were 334 beef animals from the birth cohort in Clare, and 3,016 in Galway (which is more-distant). These results confirm a widely held view of Clare as a net exporter of cattle. Conversely, Meath, a traditional cattle fattening county in the north east of Ireland, received more cattle than any of its neighbours. The dispersal of animals throughout the country has major implications for the spread of disease (Anderson, 2002; Carrique-Mas et al., 2005; Gubbins, 2005; Weber et al., 2004). In particular, as a result of rapid movement and widespread dispersal, there is the potential for rapid dissemination of infection prior to the development of clinical signs. Further work is needed to investigate the implications of movement on disease control in an Irish context. Mathematical modelling may be of particular benefit, specifically with the aim to predict the spread of infection following introduction. Other methodologies, such as network analysis (Bigras-Poulin et al., 2004; Christley et al., 2005; Webb and Sauter-Louis, 2002) may also contribute to our understanding of livestock movements and the potential for disease spread.

#### 4.2 Survival

To this point, the survival experience of Irish cattle has not been quantified. The results from this work are essentially as expected, based on our knowledge of industry practices. Survival is greatly influenced by breed and sex, with longevity being longest in female dairy cattle (cumulative probability of surviving to 4 years of age being 0.72) and shortest in male beef cattle (0.07). There is a steep decline in probability of survival for male cattle between 2 and 2.5 years of age, which concurs with known industry slaughtering practices. Dairy females show a very gradual decrease in their probability of survival throughout the study period indicating their use for production purposes (Figure 6). Female beef cattle, which mature earlier than their male counter parts, are also slaughtered at an earlier age (Figure 5). Note, however, that not as many females as males are slaughtered during this period. Survival analysis has been used previously in Austria (Essl, 1998), Italy (Samore *et al.*, 2003) and Kenya (Ojango *et al.*, 2005) to describe the changes in probability of survival of livestock.

#### 4.3 Caution

Kerry has one of the largest cattle populations of any Irish county (145,211 births registered in Kerry compared with 2.1 million nationally in 2000), and it was for this reason that it was chosen for this study. However, there are substantial regional differences in cattle management throughout Ireland, as reflected in objective measures such as the proportion of agricultural land, the density of stock, the type of land and of farms. Further, severe restrictions were placed on livestock movements throughout Ireland during 2001, at the time of the foot-and-mouth disease outbreak. This prevented all livestock from moving for a period of time, and may have altered animal movement patterns subsequently. Since the end of the study period, there have also been substantial changes to the Common Agricultural Policy of the European Union. Although there remains an incomplete understanding of the effect of these changes, these reforms are certain to affect the numbers of stock kept. As a consequence of each of these issues, the Kerry results must be extrapolated with care.

This study was only possible as a consequence of the national animal identification and tracing system managed by the Department of Agriculture and Food. The main components of the system include calf birth registration and the cattle movement and monitoring system. The resulting database is robust, given tagging and registration of all calves at birth, as well as an extensive national mechanism to trace livestock from birth to slaughter. Nonetheless, some degree of error is likely, for example data absence (for example, animals dying before being registered) or invalidity (illegal alteration of identify as a result of ear tag swapping). These issues were not considered in the current study, and will only have a substantial effect if these errors are common.

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# Critical evaluation of the true disease status of Irish cattle herds with inconclusive serological evidence of bovine brucellosis

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## Abstract

In Ireland, there has been a steady decline since 1998 in herd restrictions and depopulations 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 critically evaluate the true disease status of Irish herds and animals with inconclusive serological evidence of bovine brucellosis. During the 12 months from 1 September 2004, we collected laboratory and observational epidemiological data from all Irish herds where animal testing identified at least one animal with a CFT reading of greater than zero and/or an ELISA positive result. A total of 636 herds were enrolled. A rising CFT titre was generally associated with herds where other evidence of infection was also available. There was poor correlation between the CFT and ELISA results (positive, negative), both initially and during later assessments. The correlation between the CFT and skin test results was also poor. Based on preliminary results, the false-positive rate for the brucellin skin test in two groups of herds (each with no other evidence of infection) was estimated to be 5.4% and 42.9%, respectively. This study has highlighted the value of the CF test in the diagnosis of bovine brucellosis in Ireland. Knowledge of the CF result at the initial and a subsequent blood test has proved useful in distinguishing false positive and true positive brucellosis results. It is critical to use all available methods, both from the laboratory and the field, to maximise the accuracy of decision-making as part of the Irish national eradication programme for bovine brucellosis.

# 1. Introduction

## 1.1 Progress towards disease eradication in Ireland

A national programme to eradicate bovine brucellosis in the Republic of Ireland commenced in 1965 with the introduction of milk ring testing for dairy herds. At the outset of the programme, 12% of the 105,000 dairy herds tested positive; a further 3% of tests were inconclusive (Griffin and Collins, 1999). At this time, the incidence of disease was higher in the south of Ireland than in the west and north-west. Good progress towards the goal of eradication was achieved over the following 20 years, resulting in a recorded herd prevalence of 0.19% during 1985 and 1986 (Griffin and Collins, 1999). Residual disease was limited to north Cork, Limerick and Tipperary. However, further progress was not achieved, with disease prevalence increasing during the 1990s. A total of 1,081 herds were restricted during 1998 (Griffin and Collins, 1999), with disease spreading within counties Limerick and Tipperary, but also to counties that had been clear for a number of years. A range of policy changes were introduced from February 1998 onwards, including the re-introduction of the pre-movement test, the rapid depopulation of infected herds and the treatment of slurry with lime prior to land spreading (Figure 1). In situations where lime treatment was not possible, the herdowner was required to store the slurry or farm yard manure for a prolonged period prior to spreading. Since 1998, there has been a steady decline in herd restrictions and depopulations (Figure 2). In 2005, 144 herds were restricted as a result of confirmed or suspected brucellosis.

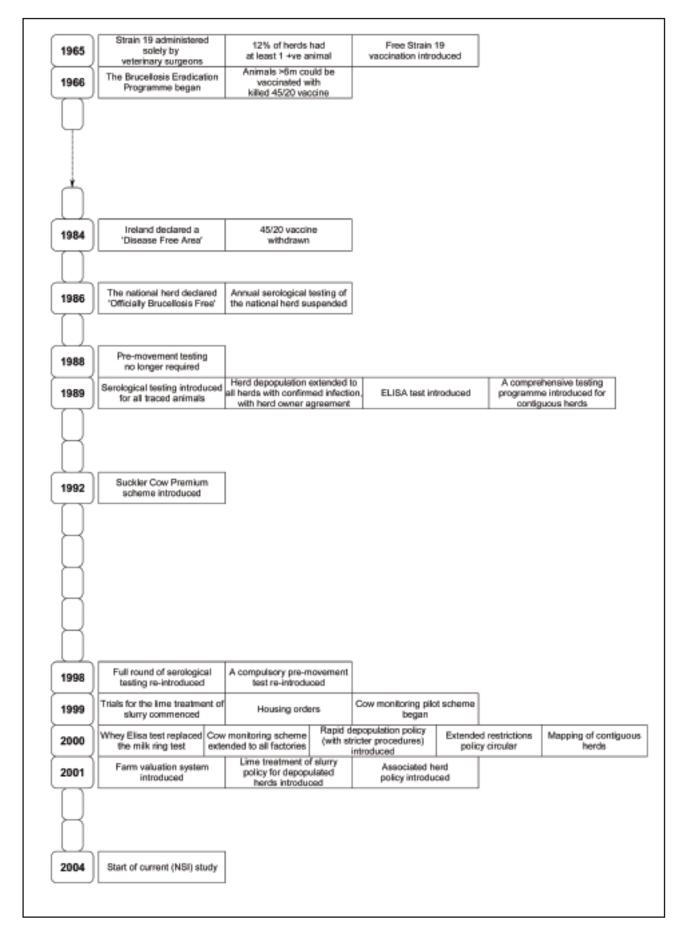


Figure 1. National policy changes, relevant to the eradication of bovine brucellosis, between 1965 and 2004

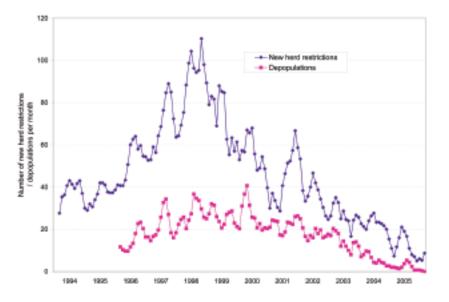


Figure 2. New herd restrictions and depopulations each month (three-month rolling average) due to bovine brucellosis in Ireland during 1994 to early 2006

#### 1.2 Challenges faced as disease prevalence falls

There is a fall in the positive predictive value of sero-diagnostic testing with reducing disease prevalence. In other words, false positive serological reactors become increasingly problematic as disease levels fall. This issue, which has been reported in the European Union and New Zealand (Pouillot *et al.*, 1998; Godfroid *et al.*, 2002) in association with the latter stages of brucellosis eradication programmes, presents a range of challenges for programme decision-makers. These include the imposition of potentially unnecessary herd restrictions, related trade implications and testing requirements for herds contiguous to those with false positive reactor animals (Godfroid *et al.*, 2002). Efforts to distinguish false and true positive reactors have been conducted, based on detailed epidemiological investigations, laboratory testing and measures of cellular immunity (Godfroid *et al.*, 2002; Saegerman *et al.*, 2004).

#### 1.3 Study objectives

The correct interpretation of test results from all female and entire male cattle over 12 months of age is a critical component of the national programme. However, the interpretation of these results may become increasingly problematic, as brucellosis prevalence falls in Ireland. The purpose of the current study was to critically evaluate the true disease status of Irish herds and animals with inconclusive serological evidence of bovine brucellosis.

## 2. Materials and methods

## 2.1 The Irish programme

As part of the national brucellosis eradication programme, blood is collected annually from all female and entire male cattle over 12 months of age for serological testing at the National Brucellosis Laboratory (Model Farm Road, Bishopstown, Co. Cork). Initially, serum is screened using the microtitre-serum agglutination test (MSAT). MSAT-positive samples are then re-tested using both the indirect enzyme linked immunosorbent assay (ELISA) and complement fixation test (CFT). If any animal returns a CFT of 19.4 international units (IU) or greater, the sera from all other eligible animals in the same herd are tested using CFT and ELISA (Figure 3). Serological testing is also conducted (in addition to the annual test) on herds that are contiguous to restricted herds, on animals pre- and post-movement, as well as on animals that have been traced from diseased premises. In addition, the whey ELISA is conducted monthly on bulk milk tank samples from all dairy herds, abortions should be submitted for detailed testing at regional laboratories, and most cull cows are also tested at point of slaughter. Movement restrictions are imposed on all test-positive herds, and rapid herd depopulation is undertaken when disease is considered to be present.

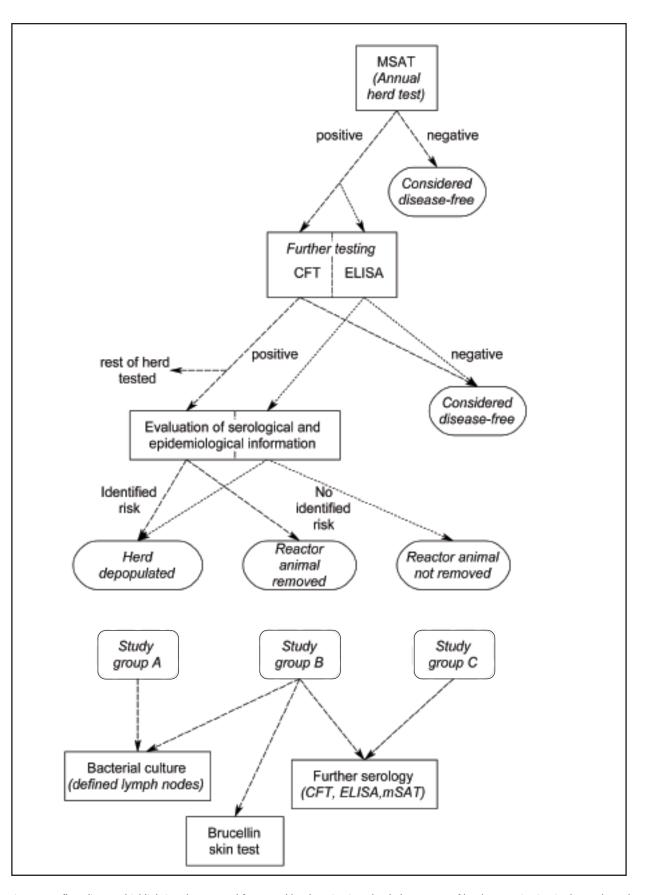


Figure 3. A flow diagram highlighting the protocol for annual herd testing in Ireland, the process of herd categorisation in the study, and the diagnostic tests applied to the different study groups

## 2.2 The data

All herds in the Republic of Ireland were eligible for inclusion in this study. We enrolled all herds into the study that showed any evidence of a serological response in either the CFT or serum ELISA at any test during the 12 months from 1 September 2004. A range of data sources were used in this study, including:

- The National Brucellosis Laboratory, which provided results from this initial test, and from all relevant testing conducted subsequently;
- The Animal Health Computer System (AHCS) and Animal Identification and Movement (AIM) System, which provide a central national database of animal movement and health information;
- The Land Parcel Identification System (LPIS), which provides geographic information on land owned by farmers; and
- Field veterinary inspectors, who conducted an epidemiological assessment of the Group B herds (see below).

#### 2.3 Categorisation of study herds

Study herds were categorised on the basis of results from the first assessment for brucellosis during the study period, as follows:

- At the initial assessment, there was confirmed evidence of brucellosis (for example, culture-positive abortion) in *Group A herds*. Alternatively, at this initial assessment Group A herds had at least two animals with a CFT result of 111 IU or more. In the latter situation, when the initial assessment was a part herd test, the result of the complete herd test was taken into account before the herd was categorised.
- At the initial assessment, there was one or more animals in *Group B herds* with a CFT reading greater than zero; however, Group A herd criteria were not met.
- At the initial assessment, there was one or more animals in *Group C herds* with a positive ELISA result; however, Group A and/or B herd criteria were not met.

Herds remained in their initial herd category, despite later events during the study period. The protocol for annual herd testing in Ireland is presented in Figure 3.

## 2.4 Further data collection

Following the annual herd test, the study herds were managed as presented in Figure 3. Briefly:

- Group A herds were immediately depopulated. When feasible, the retropharyngeal and/or supramammary lymph nodes from
  reactor animals were submitted for bacterial culture. A range of decontamination procedures were then undertaken, as
  described previously. These farms were then considered disease-free at subsequent re-population.
- In Group B herds, the study animals were re-bled for testing using the MSAT, CFT and ELISA. Where feasible, the brucellin test
  was also conducted on reactor animals at this time. Reactor animals were removed; retropharyngeal and/or supramammary
  lymph nodes were collected from these animals for bacterial culture, where feasible.
- In Group C herds, the study animals were re-bled for testing using the MSAT, CFT and ELISA. No animals were removed as part
  of disease control efforts

The disease status of each study herd will be observed until the first post-calving test after the end of the study period. These will be completed by or soon following 01 July 2006.

#### 2.5 Data collection, management and analysis

Herd and animal level data was managed in a Microsoft Access 2003 database. Data analysis was conducted in Microsoft Access, and presented using Microsoft Excel 2003. Inter-test correlation was assessed using kappa and McNemar's chi square test.

## 3. Results

#### 3.1 General results

During the 12 months from 1 September 2004, 636 herds were enrolled in the study, including 10 Group A herds (117 reactor animals), 246 Group B herds (450 animals) and 380 Group C herds (396 animals). The Group B and C herds were located throughout Ireland, whereas the Group A herds were found in counties Cavan, Clare, Cork, Kerry, Meath and Tipperary (Figure 4).

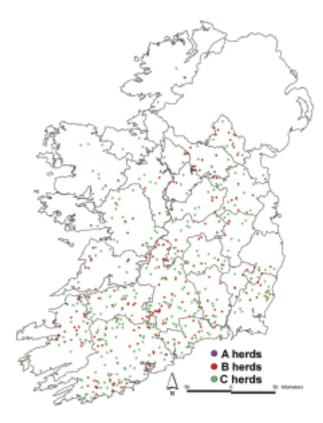


Figure 4. The location of all study herds



Figure 4a. The location of the A herds

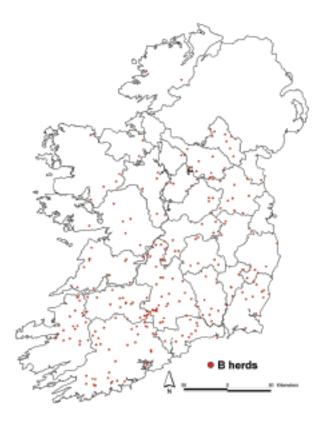


Figure 4b. The location of the B herds

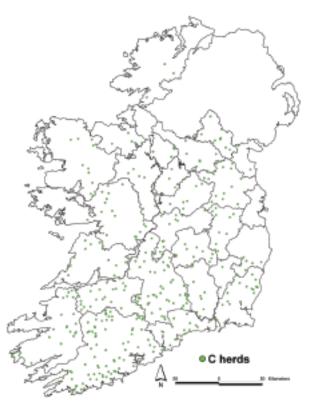


Figure 4c. The location of the C herds

## 3.2 Group A herds

Using the above-mentioned herd categorisation, 10 Group A herds were identified during the study period. Of the 117 reactor animals, 95 (81.2%) were CFT-positive and 97 (82.9%) were ELISA-positive. The distribution of the initial CFT result from these animals is presented in Table 1, and the level of agreement between the CFT and ELISA results (kappa=0.25, 95% confidence interval -0.01 to 0.50; McNemar's chi-square P = 0.69) in Table 2. Prior to depopulation, follow-up assessment was possible on 9 animals in 2 herds, including serology (CFT; 9 animals), the brucellin skin test (3) and bacterial culture of lymph notes (2). Results are presented in Table 3. At this assessment, the CFT remained high in one animal, increased in 5 and decreased in 3. No reactors have been disclosed in the re-populated herds.

Table 1. The initi study animals in C	ial CFT results from Group A herds		The CFT ar al assessmer		results from	117 animals in	10 Group A herds, at								
Initial CFT				ELISA		ELISA									
result				+ve		-ve	Total								
(IU)	No. of animals	CFT +ve	а	83(87%	6)	12(13%)	95(100%)								
0	3	CFT –ve	Ь	14(64%	6)	8(36%)	22(100%)								
13.9	4	Total		97(1009	%)	20(100%)	117								
16.7	15	a <sub>Result</sub>	<sup>a</sup> Result of 19.4 international units or greater												
19.4 4 <sup>b</sup> Result less than 19.4 IU															
28 8															
33 1 Table 3. The initial and subsequent test results from animals in Group A herds															
39	8	where s	ubsequent te	esting was cor	nducted										
44	1		Ar	nnual herd test	t Su	bsequent assessn	nent								
56	5			CFT (IU)	CFT (IU)	Brucellin test	Culture								
67	7					(mm increase)									
78	3	Herd 1	Animal 1	356	356										
111	4	Herd 1	Animal 2	16.7	78		Positive								
133	3	Herd 1	Animal 3	67	33		Negative								
156	2	Herd 1	Animal 4	78	356		regutive								
178	4	Herd 1	Animal 5	266	111										
222	6	Herd 2	Animal 1	39	67	0									
266	1	Herd 2	Animal 2	19.4	156	4									
311	3	Herd 2	Animal 2 Animal 3	39	222	4									
356	34	Herd 2	Animal 4	356	222	U									
Haemolysed	1	Tierd Z	Anninat 4	550											

## 3.2 Group B herds

In the Group B herds, initial serological data are available for 440 animals from 246 herds (Table 4; samples from 10 animals were haemolysed and could not be tested). These results include all animals in the Group B herds with serological responses to the ELISA and/or CFT, including (but not restricted) to those with a CFT of 19.4 IU or greater. At this assessment, 225 (51.1%) samples had a CFT reading greater than zero, and 330 (75.0%) samples were ELISA-positive. There is a poor correlation between the initial CFT and ELISA results (kappa = -0.38, 95% confidence interval -0.47 to -0.30; McNemar's chi square test P < 0.001).

Table 4. The CFT ar	nd ELISA test results fro	om 440 animals in 24	46 Group B herds, at
the initial assessm	ent. Samples from a fu	rther 10 animals we	re haemolysed and
could not be teste	d		
	ELISA +ve	ELISA -ve	Total
CFT +ve <sup>a</sup>	127	98	225
CFT –ve <sup>b</sup>	203	12	215
Total	330	110	440 <sup>a</sup>
2			
	ernational units or great	ter	
<sup>b</sup> Result less than 1	9.4 IU		

Follow-up data, including herd-level risk factor information and diagnostic results were collected on 334 animals in 198 herds (Figure 5). The CFT results for 333 animals (sera from one animal was haemolysed) at the initial and a subsequent assessment are presented in Table 5. ELISA results are available for 292 of these animals on two occasions (Table 6), and from 42 on three occasions (Table 7). The

brucellin test was conducted on 221 animals, and bacterial culture on 93 animals (Figure 5). The correlation between the CFT result at the first and subsequent assessment is presented in summary form (Table 5) and in detail (Table 8). The correlation between the initial and subsequent CFT was poor (kappa = 0.26, 95% confidence interval 0.16 to 0.36; McNemar's chi square test P < 0.001), mainly due to a falling CFT result at the subsequent test. Of 173 animals with a positive CFT reading at the initial assessment, 122 (70.5%) subsequently tested negative (Table 5). There was poor correlation between the initial and subsequent ELISA results (Table 6; kappa = -0.37, 95% confidence interval -0.52 to -0.26; McNemar's chi square test P = 0.20), and poor correlation between the CFT and ELISA results at the subsequent test, of the 56 animals with a CFT reading of greater than zero, 38 (67.9%) were also ELISA-positive. The correlation between the CFT and skin test results was poor (kappa = 0.03, 95% confidence interval -0.21 to 0.27; McNemar's chi square test P < 0.001.), (Table 10). In total, 12 animals (5.4% of 221 tested) were positive to the brucellin skin test; 3 (25.0%) also had a positive CFT reading. One animal was positive at culture, of 93 submitted (Figure 5).

Table 5. Summary of the CFT result at the initial and subsequent assessment for 333 animals in 198 Group B herds. Paired sera were available for one further animal, however, sera taken at the subsequent assessment was haemolysed and could not be tested

At initial assessment	At subsequen	it assessment	
	CFT +ve <sup>a</sup>	CFT -ve	Total
CFT +ve <sup>a</sup>	51	122	173
CFT –ve <sup>b</sup>	5	155	160

56

277

<sup>a</sup> Result of 19.4 international units or greater <sup>b</sup> Result less than 19.4 IU

Total

 Table 6.
 ELISA results from 292 animals in Group B study

 herds, where data from the initial and at a subsequent

 assessment are available

ELISA t	est result	
At initial assessment	At subsequent assessment	Number (%) of animals
Positive	Positive	113 (38.7)
Positive	Negative	96 (32.8)
Negative	Negative	79 (27.0)
Negative	Positive	4 (1.4)

#### Table 7: ELISA results from 42 animals in Group B study herds, where data were available from three ELISA assessments

333

	ELISA test result		Number (%) of animals				
Result at first test	Result at second test	Result at third test					
Positive	Positive	Positive	20 (47.6)				
Positive	Positive	Negative	6 (14.3)				
Positive	Negative	Positive	4 (9.5)				
Positive	Negative	Negative	6 (14.3)				
Negative	Positive	Positive	0				
Negative	Positive	Negative	1 (2.4)				
Negative	Negative	Positive	0				
Negative	Negative	Negative	5 (11.9)				

Table 8. CFT result at the initial and subsequent assessment for 334 animals from 198 Group B herds.

	FT result	Numerican				CF	resu	ılt at	subs	eque	nt as	sessr	nent	(IU)									
	at initial ssment (IU)	Number of animals	0	13.9	16.7	19.4	22.2	28	33	39	44	56	67	78	89	111	133	156	178	222	266	311	356
	0	91 <sup>a</sup>	83	4		2	1																
	13.9	46	35	9	2																		
	16.7	24	16	5	1	1					1												
	19.4	32	20	7	3	1		1															
	22.2	7	4	1	2																		
	28	15	7	4	3					1													
	33	19	11	3	1			2	1		1												
	39	17	6	2	4	1		1	1	1													1
	44	6		1	1	1		2		1													
	56	22	6	6	2	2		1	2			1					1						1
	67	16	5	3	2				1		1		1			1			1			1	
	78	8	2	3					2		1												
	89	5		2	1					1			1										
	111	9	3	2	1				1	2													
	133	7	2		1				2		1					1							
	156	1								1													
	178	2														1							1
	222	1																					1
	266	1								1													
311		1 1																					
356		4											1										3

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 Table 9.
 The CFT and ELISA test results from 333 animals in 246 Group B herds, at the subsequent assessment. A haemolysed serum sample from one further animal could not be tested

	ELISA +ve	ELISA -ve	Total	
CFT +ve <sup>a</sup> CFT –ve <sup>b</sup>	38	18	56	
CFT –ve <sup>b</sup>	103	174	277	
	141	192	333	

<sup>a</sup> Result of 19.4 international units or greater

<sup>b</sup> Result less than 19.4 IU

Table 10. The CFT and brucellin skin test results from 220 animals in Group B herds at the subsequent assessment. The CFT result from one animal (brucellin skin test reading of 0 mm) was not determined, because the serum was haemolysed

Brucellin skin test +ve	Brucellin skin test -ve	Total	
3	39	42	
9	169	178	
12	208	220	
	3 9	3 39 9 169	3         39         42           9         169         178

<sup>a</sup> Result of 19.4 international units or greater

<sup>b</sup> Result less than 19.4 IU

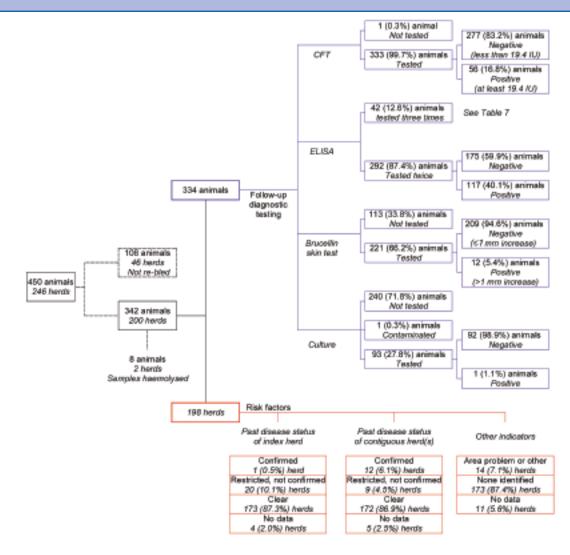


Figure 5. Risk factors and follow-up diagnostic test results for 334 animals in 108 Group B herds

#### 3.4 Group C herds

In the Group C herds, 396 animals from 380 herds were ELISA-positive at the initial blood test, but no further Group A or B herd criteria were met. Follow-up data, including herd-level risk factor information and serological results were collected on 248 of these animals in 240 herds (Figure 6). At the subsequent assessment, there were two (0.8%) animals with a positive CFT reading (Figure 6) and 68 (39.1%) ELISA-positive animals (Figure 6, Table 11).

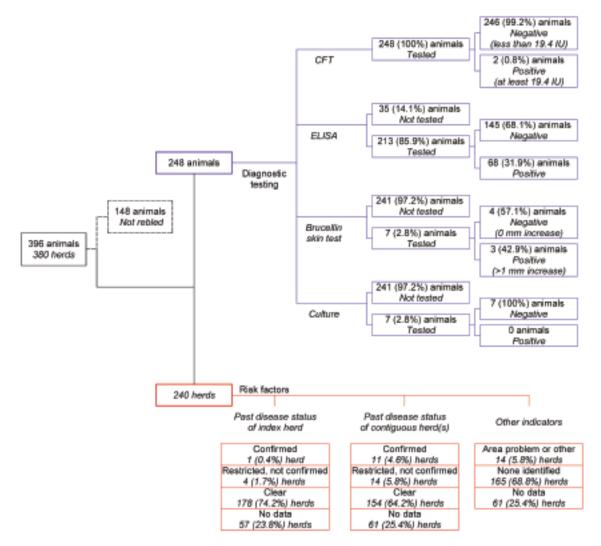


Figure 6. Risk factors and diagnostic test results for 248 animals in 240 Group C herds

Table 11. ELISA results from 213 animals in Group C study herds, where data from the initial and at a subsequent assessment are available

est result	
At subsequent assessment	Number (%) of animals
Positive	68 (31.9%)
Negative	145 (68.1%)
	At subsequent assessment Positive

The status of these herds at the post-calving test is pending.

#### 4. Discussion

In this study, we have sought to evaluate the true disease status of Irish herds and animals with inconclusive serological evidence of bovine brucellosis. This assessment was essentially observational, relying on field and laboratory data collected as part of (and in some cases additional to) the nationally-directed disease eradication programme. For some years, rapid herd depopulation has been an impor-

tant component of the national programme, in situations where infection is likely. Consequently, during this study it was often not possible to conduct exhaustive laboratory tests to directly determine the true disease status of these herds and animals. Rather, we have used a range of methods to indirectly assess this, including the disease status of the index, contiguous and associated herds from the time of the initial test through to the subsequent post-calving test.

As yet, we have not yet been able to determine the true disease status of each of the study herds. Based on all available information, there is no evidence to suggest that any of the 380 Group C herds had been infected during the study period. Although low CFT positive readings were subsequently detected in two of these herds, no further reactors have been disclosed. Because the post-calving tests are not yet complete, it is not possible to determine the percentage of Group B herds that were infected with *B. abortus*. The ten Group A herds were all rapidly depopulated following initial diagnosis, based on compelling laboratory and/or epidemiological evidence that infection was present. A rigorous programme of post-infection decontamination was subsequently conducted in each of these herds, including the addition of lime into the slurry tank. Each of these A herds has subsequently restocked, and no reactors have subsequently been disclosed.

This study confirms the value of the CFT in the diagnosis of bovine brucellosis. High CFT results and substantial increases in the CFT result at sequential blood tests were each associated with confirmed disease. Of the 248 study animals in C herds, 2 (0.8%) are believed to have falsely tested positive (Figure 6). In B herds, the CFT results generally dropped at a second bleed (Table 8, Figure 5), which was mainly conducted within one month of the initial test. Similar patterns of results have been attributed to infection with cross-reacting organisms. In general, when cross reactions occur, only one animal or a very small proportion of the herd is affected. Further, serological titres in these animals usually drop within a few weeks (Corbel *et al.*, 1984). The specificity of the CFT test may be compromised when animal populations are infected with *Yersinia enterocoliticia* serotype 0:9 (Godfroid *et al.*, 2002). Godfroid et al. (2002) have found that the different brucellosis serological tests could not differentiate brucellosis from *Y.enterocolitica* serotype 0:9 infection, whereas the skin test could. Work is currently underway in Ireland to clarify the importance of cross-reacting organisms in animals with transient CFT results. Findings from the present work on *Y.enterocolitica* serotype 0:9 isolation will assist in quantifying this problem, and also in further evaluating the value of the skin test in the lrish context.

In this study, there was poor correlation between the CFT and ELISA results, particularly among study animals in B herds. At the initial bleed, the level of agreement (measured using kappa) between the CFT and ELISA results from study animals in Group A herds was 0.25. The equivalent statistic for study animals in Group B was -0.38; that is, the strength of agreement was worse than expected by chance alone. Further, the reduction in antibody response was more pronounced with the CFT (36.6% of samples had a CFT reading of 19.4 IU or greater at the first assessment, but less than 19.4 IU at the second assessment; Table 5) as compared with the ELISA (32.8% samples were positive at the first assessment and negative at the second; Table 6). In broad terms, these results suggest that the ELISA was more sensitive and less specific than the CF test. Further, a reduction in CFT results at a second assessment may provide greater evidence of disease freedom than a reduction in ELISA results. However, the relationship is not straightforward, given the substantial number of CFT-positive, ELISA-negative study animals from the A (10.2%; Table 2) and B (22.2%; Table 4) herds at the initial assessment. Guidelines may be needed in relation to the use of the ELISA and for its interpretation in different situations.

Based on international reports, the skin test is considered highly specific (greater than 99%) with moderate to high sensitivity (64-93%) (Pouillot *et al.*, 1997; Saegerman *et al.*, 1999; Bercovich and Muskens, 1999). Based on work by Saegerman *et al.* (1999), there is a drop in sensitivity with increasing time following infection. In the current study, the brucellin skin test was conducted on 221 (66.2%) study animals from the Group B herds and 7 (2.8%) study animals from the Group C herds. Assuming that each of these animals was not infected, the false-positive rate in the B and C herds was 5.4% and 42.9%, respectively. There was also poor agreement between the CFT and skin test results in study animals from Group B herds (Table 10). These results are disappointing, given the international experience, for reasons that are not currently apparent. Once the results of the post-calving test are known, it will be possible to have greater confidence in the true disease status of the Group B herds.

As part of this study, data on a range of epidemiological risk factors was collected from each of the B and C herds, including the past disease status of the index and contiguous herds, and other potential indicators of infection including the presence (or otherwise) of infection in the locality. The distribution of these risk factors between B and C herds is not dissimilar, and may reflect – at least in part – the historically widespread nature of infection in Ireland. For example, in 6.1% and 4.6% of Group B and C herds, respectively, there is a past history of disease in one or more contiguous herds. Among the Group A herds, and those Group B herds where disease was subsequently suspected or confirmed, a combined understanding of all relevant information (including epidemiological risk factors) proved critical to effective decision-making.

In conclusion, this study has highlighted the value of the CF test in the diagnosis of bovine brucellosis in Ireland. Knowledge of the CF result at the initial and a subsequent blood test has proved useful in distinguishing false positive and true positive brucellosis results. Based on the current results, the ELISA test appears to be of lesser value. Recent findings confirm the presence of infection with *Yersinia enterocolitica* serotype 0:9 in some Irish herds, which may be leading to false-positive CFT reactions. Reliance should not be placed on serology alone, noting that non-pregnant heifers may give a weak and transient response to a challenge with brucella (Cunningham,

1968; Wilkinson *et al.*, 1988). Pregnant heifers may present a similar serological picture (Cunningham, 1968). The study has highlighted the importance of epidemiological investigations as part of any investigation, to ascertain potential linkages with known (or suspected) sources of infection. Therefore, it is critical to use all available methods, both from the laboratory and the field, to maximise the accuracy of decision-making as part of the Irish national eradication programme for bovine brucellosis.

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# Irish herds derestricted during 2000 following a period of restriction due to brucellosis: descriptive analysis of data collected by attending Veterinary Inspectors

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## Abstract

In Ireland, progress is being made towards national eradication of bovine brucellosis. As part of the national programme, data are collected by attending Veterinary Inspectors (VI) at the point of herd derestriction. This study concerns the analysis of data collected from herds that were derestricted during 2000, following a period of restriction due to brucellosis. The objectives of this study included the spatial and temporal distribution of derestricted herds, initial methods of disease detection, the source of disease (in the VI's opinion), the use of microbiological confirmation, and the subsequent disease history of diseased herds that were not depopulated. Data were collected from 574 (71.2%) herds at the point of derestriction. The case herds were clustered south of the River Shannon. Dairy herds predominated. Most of the herds had been restricted following a positive serological test; in 57.6% of these herds there had been a single serological reactor. In total, microbiological culture had been conducted on 42.3% herds, 41.6% of these were culture positive. In the opinion of the attending VI, local spread (residual, adjacent, associated or mechanical) was the probable source of infection. The study highlights challenges associated with the interpretation of serological results.

## 1. Introduction

A national programme to eradicate bovine brucellosis from the Republic of Ireland commenced in 1965. In 1965, based on results of the Milk Ring Test of milk samples from 105,000 dairy herds, 12% of herds were positive and 3% inconclusive. The incidence of disease was higher in the south of the country than in the west and north-west. During the following 20 years, there was good progress towards national eradication. In 1985 and 1986, 191,234 herds were tested serologically for brucellosis , 368 were restricted, and 184 depopulated (herd prevalence of 0.19%). These herd restrictions were limited to North Cork, Limerick and Tipperary. By 1992, however, there was some deterioration in the national picture, resulting in an increase in the number of restrictions in Limerick and Tipperary, as well as restrictions in counties that had been clear for a number of years. 327 herds were restricted during that year. The situation continued to worsen until 1998, with 1,081 new herd restrictions in that year (Griffin and Collins, 1999). Improved disease control measures were introduced, resulting in a steady decline in the prevalence of disease between 1999 and 2004. In 2004, there were 323 new herd restrictions, including 51 herd depopulations (herd prevalence of 0.04%).

The brucellosis eradication programme in Ireland involves the serological testing each year of all female cattle and bulls over one year of age. Serological testing is also conducted on contiguous herds, on animals pre- and post-movement, and on animals traced from diseased premises. Whey ELISA test are carried out on bulk milk tank samples, and all abortions are notifiable. Cull cows are also tested at point of slaughter. Controls include the imposition of movement restrictions and full herd depopulations, if warranted.

Data are routinely collected at the point of herd derestriction, with the aim to provide information to provide information about aspects of the eradication scheme, and, potentially, to influence policy into the future. As yet, however, these data have not been analysed. This study concerns the analysis of data collected from herds that were derestricted during 2000, following a period of restriction due to brucellosis. The objectives of this study included the spatial and temporal distribution of derestricted herds, initial methods of disease detection, the source of disease (in the opinion of the attending Veterinary Inspector), the use of microbiological confirmation, and the subsequent disease history of diseased herds that were not depopulated.

## 2. Material and methods

At the point of derestriction, data were gathered on farm by a district Veterinary Inspector using a purpose-built questionnaire. The questionnaire covered a range of issues relating to the herd restriction, including the herd number and location of the derestricted herd, the method of initial disease identification, the source of disease (in the opinion of the Veterinary Inspector), whether *Br. abortus* had been isolated during the outbreak, and the subsequent disease history of diseased herds that were not depopulated. Data about each of these herds (case herds) were entered into a database (Epi Info version 6.04, Centers for Disease Control and Prevention, Atlanta GA, USA), and subsequently transferred to Microsoft Access 2000 (Microsoft, Redmond WA, USA) for analysis. Graphs created using Microsoft Excel. Spatial distribution maps were produced using ArcView (ESRI, Redlands CA, USA). The risk density maps were produced using the Kernel Smoothing technique, with a kernel bandwidth of 8km (Bowman and Azzalini, 1997).

## 3. Results

## 3.1 The case herds

Approximately 800 herds were derestricted during 2000 following a brucellosis-related restriction. Data were collected from 574 (71.2%) of these herds at the point of derestriction.

Of the 574 case herds, there were 301 (52.4%) dairy herds, 219 (38.2%) are suckler herds, 38 (6.6%) combined enterprises and 16 (2.8%) feeder farms. Of the farmers whose status is known, 233 (82.0%) were full time farmers and 51 (18.0%) were part-timers. The number of cattle on each premises ranged from one animal to 631 animals, with a median of 66 cattle.

The case herds were mainly clustered in a band from the River Shannon to the southwest, including Limerick (133 herds; 23.2%), Kerry (99; 17.2%), Tipperary North (100; 17.4%) and Cork South (59; 10.3%) (Table 1, Figure 1). The geographic risk density is presented in Table 2

	1. The number of herds derestricted following losis restriction during 2000, by county				
County	Number	Percentage			
Carlow	0	0.0			
Cavan	11	1.9			
Clare	21	3.7			
Cork N	31	5.4			
Cork South	59	10.3			
Donegal	0	0.0			
Dub/Wick E	4	0.7			
Galway	16	2.8			
Kerry	99	17.2			
Kild/Wick W	1	0.2			
Kilkenny	8	1.4			
Laois	12	2.1			
Leitrim	0	0.0			
Limerick	133	23.2			
Longford	1	0.2			
Louth	0	0.0			
Mayo	3	0.5			
Meath	0	0.0			
Monaghan	0	0.0			
Offaly	36	6.3			
Roscommon	9	1.6			
Sligo	0	0.0			
Tipperary N	100	17.4			
Tipperary S	25	4.4			
Waterford	5	0.9			
Westmeath	0	0.0			
Wexford	0	0.0			
Wicklow	0	0.0			

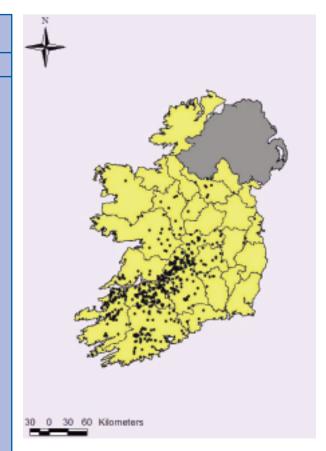


Figure 1. The location of 574 herds that were derestricted following brucellosis restriction during 2000

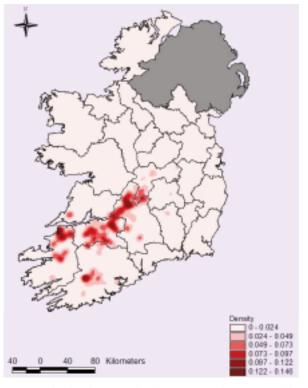


Figure 2: The distribution of herd restriction risk per square kilometre, based on data from 574 herds that were derestricted following brucellosis restriction during 2000. The intensity of colour is illustrative of the level of herd restriction risk.

# 3.2 The restrictions

The case herds were restricted between March 1998 and December 2000, with most occurring between June 1999 and June 2000. The median length of restriction was 185 days, with a minimum of 23 and a maximum of 802 days. Fifteen herds were excluded from these latter calculations, due to invalid dates of restriction and de restriction.

These herds were restricted for a range of reasons. A total of 469 (81.7%) of the herds were restricted following positive serological tests (Table 2); the balance were restricted on the basis of routine testing of whey (56; 9.8%), and following notification of an abortion (49; 8.5%). In 269 (57.6%) of the 469 herds that tested positive based on initial serological testing, there was a single reactor only. Samples were cultured from 143 of these latter herds, with 37 (25.9%) culturing positive.

Table 2: The method of initial detection, leading to restriction of the 574 case herds.			
Method of disease identification	Number of case herds	% of case herds	
Whey ELISA	56	9.8	
Positive abortion	49	8.5	
Routine serological testing			
Tracing	6	1.0	
Round Test	168	29.3	
Contiguous Test	211	36.8	
SCT	16	2.8	
Pre-Movement	28	4.9	
Post-Movement	9	1.6	
Inconclusive Retest	31	5.4	

Two hundred and forty three (42.3%) herds had lymph nodes collected and sent to the laboratory for bacterial isolation and culture; 101 (41.6%) of these herds were culture positive. Following a culture positive abortion, 95% of herds submitting lymph nodes returned a positive result.

In total, 235 (40.9%) case herds were depopulated fully; a further 14 (2.4%) were partially depopulated. Full-herd depopulations were not conducted on 36 herds in Co. Clare, Cork, Galway, Kerry, Limerick, Offaly and Tipperary, where a total of 56 animals were culture positive for *Br. abortus*. These animals included 44 (78.6%) cows, 1(1.8%) pregnant heifer and 11 (19.4%) non-pregnant heifers. Subsequently, 28 (77.8%) of these herds were negative at later herd testing, 3 (8.3%) herds had reactors during herd testing between 2001 and 2004, and 5 (13.9%) herds were lost to follow up.

#### 3.3 The attending Veterinary Inspector

In the opinion of the relevant Veterinary Inspector, 367 (63.9%) of the case herds were likely diseased. One hundred and eighteen (20.5%) of these herds were not depopulated. In 136 (37.1%) of these 367 herds, lymph nodes were submitted for culture; 99 (72.8%) were culture positive. Of the 469 herds restricted following positive serological tests, 270 (57.6%) were considered diseased by the attending Veterinary Inspector.

The source of infection for these 367 case herds, based on the opinion of the Veterinary Inspector, is presented in Table 3.

Table3. The source of infection for 367 case herds, based on the opinion of the attending Veterinary Inspector. This table excludes 207 case herds where, in the opinion of the Veterinary Inspector, infection was not present

Source	Nos. of Cases	% of case herds
Purchased	28	7.6
Residual	11	3.0
Neighbour	196	53.4
Adjacent	42	11.4
Associated	23	6.3
Mechanical	17	4.6
Unknown	50	13.6

## 4. Discussion

There are many challenges associated with the eradication of bovine brucellosis from a national herd. Brucellosis is highly infectious, with the potential for rapid within-herd transmission and high levels of environmental contamination. Further, infected animals can remain latently infected for some years. Consequently, rapid response to suspected and confirmed disease is critical to eradication success. Because diagnostic confirmation can be problematic, the Irish programme relies on the rapid imposition of movement restrictions on all suspected cases, followed by animal or full herd depopulations in herds where infection is likely. Therefore, decisions often need to be taken rapidly, on the basis of imperfect information. The current study seeks to expand our understanding of the national programme, providing some reflection on the accuracy of decision making, based on the observations and opinions of attending Veterinary Inspectors at the time of herd derestriction. These perspectives have not previously been reported from the Irish programme.

These results, which include a summary of the opinion of the attending Veterinary Inspectors, must be interpreted with some care. These people have a detailed understanding of the local situation, and most also have extensive experience in the practical control of bovine brucellosis. Further, steps were taken – using a standardised questionnaire – to maximise the uniformity of data collection. Nonetheless, all relevant information may not have been available at the time these questionnaires were completed. For example, farmers may have neglected to mention extended family connections, casual workers etc. In addition, data were collected from 574 of the 800 or so herds that were de-restricted during 2000. Herd enrolment was dependent on the interest of both the attending Veterinary Inspector and relevant farmer, which may have resulted in some level of selection bias. No data were available on the non-enrolled herds; therefore, the level of bias could not be assessed.

Based on the opinion of attending Veterinary Inspectors, local spread (residual, adjacent, associated or mechanical; Table 3) was the probable source of infection on most case herds. These opinions appear somewhat at odds with the actual method of detection. Round testing accounts for almost 30% of all restrictions, and a further 9.7% of restrictions were first detected through the Milk Ring Test. In other words, the testing results suggest that a substantial number of restrictions were first detected in herds where disease was not previously suspected. On balance, however, it is likely that local spread is of much greater significance that is reflected based on testing information alone. The attending Veterinary Inspectors base their opinion on information from a wide range of sources. Further, with this additional information, the Veterinary Inspectors will likely be better able to more accurately distinguish true positive and false positive test results. Problems with the accuracy of test results are discussed below. Supporting the above-mentioned view, the national picture is of a highly clustered disease as a result of local spread, with brucellosis risk being highest in Limerick, northeast Kerry, northern Tipperary and central Cork. Combined, these findings provide strong validation for the ongoing policy of rapid herd depopulation. In situations where local spread is important, rapid depopulation provides an effective means to rapidly reduce or prevent ongoing transmission of infection.

This study highlights some of the challenges associated with the interpretation of serological results. In the opinion of the attending Veterinary Inspector, infection was probably present in 270 (57.8%) of the 467 herds that were restricted following positive serological tests (that is, excluding herds restricted on the basis of milk testing and abortion notifications). This judgement was made at the point of derestriction, by which time a range of evidence concerning the true disease status of these herds should have been available. In the Irish programme, it is appropriate that a cautious approach is taken when positive serological results are obtained, noting the urgency generally associated with decision making. Further, a single sero-positive test result should not automatically be considered a false-positive reaction. In this study, bacterial confirmation of infection was obtained from 37 (25.9%) of 143 herds with a single reactor where further laboratory testing was conducted. It is important to highlight that the positive predictive value of diagnostic testing is closely linked with the prevalence of disease (Martin *et al.*, 1997); as disease prevalence drops, it is likely that this issue will become increasingly problematic.

Given our understanding of the epidemiology of brucellosis, it is surprising that no further problems were identified in the 36 herds where infection was confirmed but full herd depopulations were not conducted. Among 28 (77.8%) of these herds there was no further evidence of infection. It must be assumed that there had been no opportunity for within-herd transmission prior to the removal of all infected animals.

This analysis highlights the value – but also the under-utilisation – of microbiological testing as part of the national programme during this time period. Lymph nodes were collected for culture from only 37.1% herds where – in the opinion of the attending Veterinary Inspector – disease was thought to be present. Further, lymph nodes were collected, and bacterial culture conducted, on 42.3% of the case herds in this study. Based on the data available, it is not possible to determine the true sensitivity of bacterial culture, however, it is certainly higher in herds with active infection. In herds with at least one abortion, 95% of lymph nodes returned a positive result. In herds restricted following a positive serological results and where the attending Veterinary Inspector considered infection to be present, the proportion of culture positives was substantially less than this. In order to maximise information available for national and local decision-making, it is recommended that lymph nodes are collected for brucella isolation and culture, whenever feasible.

In this study, a greater number of dairy as compared to beef herds were derestricted during 2000. It is likely that the incidence of brucellosis (based on the measures of new herd restrictions and herd depopulations) was also higher in dairy as compared to beef animals, after considering the number of dairy animals in each of the key counties (diary animals represented 50.2, 47.3, 46.3 and 39.3% of all cattle in Counties Cork, Kerry, Limerick and Tipperary at the end of 2002; Anon., 2002) and the larger number of animals – on average – in dairy than beef herds. Nonetheless, brucellosis in suckler herds represents its own challenges, including level of interaction between the herdkeeper and his/her stock and the heavy reliance on serological testing and abortion notification for the detection of infection. Therefore, suckler herds may play an important role in the maintenance and spread of disease.

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# An outbreak of bovine brucellosis in county Clare, Ireland, during 2005

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## Abstract

There has been a progressive drop in the herd incidence of brucellosis in Ireland since 1998. In the latter stages of the eradication programme, information from epidemiological field investigations provide an opportunity to continually evaluate the effectiveness of existing disease control efforts. This paper describes an investigation of an outbreak of bovine brucellosis in County Clare, Ireland, during 2005. The source of infection could not be determined. Following introduction, within-herd transmission was rapid, facilitated by a range of factors, including winter housing (close contact) of cattle and the ongoing mixing of animals (both during grazing and at housing) throughout the year. Disease containment, including the near-absence of contiguous spread, was greatly facilitated by the recent construction of a shed for winter housing.

## 1. Introduction

There has been a progressive drop in the herd incidence of brucellosis in Ireland since 1998 (Sheahan *et al.*, 2006). During 2005, there were 144 new herd restrictions and 27 herd depopulations. There has also been a steady improvement in the brucellosis situation in Northern Ireland (DARDNI, 2006), following a decline in cases from 2003. With ongoing progress in both jurisdictions, it is hoped that bovine brucellosis can be eradicated from the island of Ireland.

In general terms, disease outbreak investigations are undertaken to halt disease progress, identify reasons for the outbreak, institute corrective measures and recommend procedures to reduce the risk of future outbreaks (Martin *et al.*, 1987). During the latter stages of a disease eradication programme and as part of these broader objectives, investigations offer the opportunity to gain a detailed understanding of disease behaviour. With this information, it is possible to focus disease control efforts on those factors that continue to promote ongoing disease spread. A number of reports are available concerning field investigations to clarify the behaviour of bovine brucellosis as part of (or prior to the establishment of) national eradication programmes, including work in Ghana (Kubuafor *et al.*, 2000), Syria (Darwish and Benkirane, 2001) and the USA (Ferrari and Garrott, 2002). This paper describes an investigation to determine and describe the source and spread of infection during an outbreak of bovine brucellosis in County Clare, Ireland, during 2005.

# 2. The farm

The index farm was located in County Clare. The farm specialised in suckler production and was managed over three separate land fragments. At the time of the outbreak, there were approximately 60 cows, 20 calves and 15 bullocks present on the farm. During spring to autumn each year, cattle were grazed mainly on the home (main) fragment. There was considerable mixing of cattle whilst grazing. Between December and March each year, animals were shedded at three different locations (sheds A to C), each located on the home fragment. Sheds A and B were very old, each with earthen floors and no internal partitions. Shed C was near-new, with slatted concrete floors and the potential for 9 separate cattle pens. The farm has year-round calving, with some concentration during April and May. This part of Co. Clare is considered an 'outgoing' cattle producing area (cattle introductions have traditionally been rare).

## 3. Key events

At a private test on 13 April 2005, positive serological results were detected in 3 of 10 2004 born heifers presented for a private test (animal 0283: CFT 19.4 IU; EIA -ve; 0279 & 0281: CFT 39 IU, EIA +ve). Further serological testing was conducted on 25 April 2005 (the three heifers that were seropositive on 13 April 2005) and 28 April 2005 (a full-herd test of 59 animals, including the 7 heifers that were seronegative on 13 April 2005). At these tests, 33 of 62 cattle were CFT +ve, as follows:

- 2004-born heifers: 5 of 14 positive, including the previous three [heifer number 0279 (CFT 222 IU, EIA +ve, MSAT 4/80), 0231 (CFT 67 IU, EIA -ve, MSAT 4/20), 0283 (CFT 156 IU, EIA +ve, MSAT 4/40)] and 2 additional [0268 (CFT 44 IU, EIA +ve), 0286 (CFT 19.4 IU, EIA -ve).
- 2002-born cow: 1 of 2 positive (CFT result 78 IU)
- 2001-born cows: 3 of 4 positive (+ve CFT results 356, 111, 39 IU)
- 2000-born cows: 2 of 4 positive (+ve CFT results 356, 39 IU)

- 1999-born cows: 2 of 3 positive (+ve CFT results 222, 222 IU)
- 1998-born cows: 0 of 1 positive
- 1997-born cows: 2 of 6 positive (+ve CFT 356, 356 IU)
- 1996-born cows: 6 of 6 positive (+CFT 222, 67, 39, 28, 28, 28 IU)
- 2004-born bull: 0 of 1 positive
- 2000-born bull: 1 (of 1) positive (CFT 16.7 IU, EIA +ve)

A timeline of events on the farm from June 2004 to May 2005 is presented in Figure 1. Prior to the disease outbreak, a full-herd test for brucellosis had last been conducted on 11 June 2004. In the following 12 months, animals had moved from the farm on five occasions (in 2004: 24 June, 29 June, 6 September, 16 November; in 2005: 24 February). There was a single introduction onto the farm during this period, a 2 week old calf on either 24 or 26 February 2005. All cattle were moved into housing during December 2004, and subsequently remained indoors throughout winter. Shed A held approximately 10 older cows, shed B male animals only, and shed C the balance. According to the herd-owner, there were few signs consistent with brucellosis prior to late April 2005. One of the cows in shed A (ID unknown) suffered substantial weight loss from December 2004. In late February/early March 2005, signs of heat were noticed in another cow (ID unknown) in this shed. On 24 February 2005, a group of 11 animals (including six 2002/03-born heifers that had been held throughout winter in shed C) were sold (see Figure 2). The heifers were all serologically-negative to brucellosis at a private test. The cows in shed A were all moved for calving to shed C in early March 2005 (Figure 3). Ten 2004 born heifers were moved from shed C in late March, with 3 of these animals testing positive to brucellosis at a private test. An abortion was observed in shed C on 24 April 2005 (cow number 009: a 1994-born cow previously held in shed A; CFT 356 IU at post-abortion test on 25 April 2005, CFT 222 IU at full-herd test on 28 April 2005). Following the serological testing on 25 and 28 April 2005 (with 33 of 62 animals positive), one further abortion was detected on 04 May 2005 (011: a 1994-born cow previously held in shed A; CFT 28 IU at full-herd test on 28 April 2005). The herd was depopulated on 5 May 2005. There was evidence of onward spread to one contiguous farm, with a single heifer in a herd of 14 cows testing positive (CFT 23 IU, EIA +ve) on 6 February 2006. This animal was slaughtered. Subsequent tests on this herd on 20 February and 25 April 2006 have been clear. At the latter test, 5 of the cows had recently-calved. All trace-forward investigations from the index farm were negative.

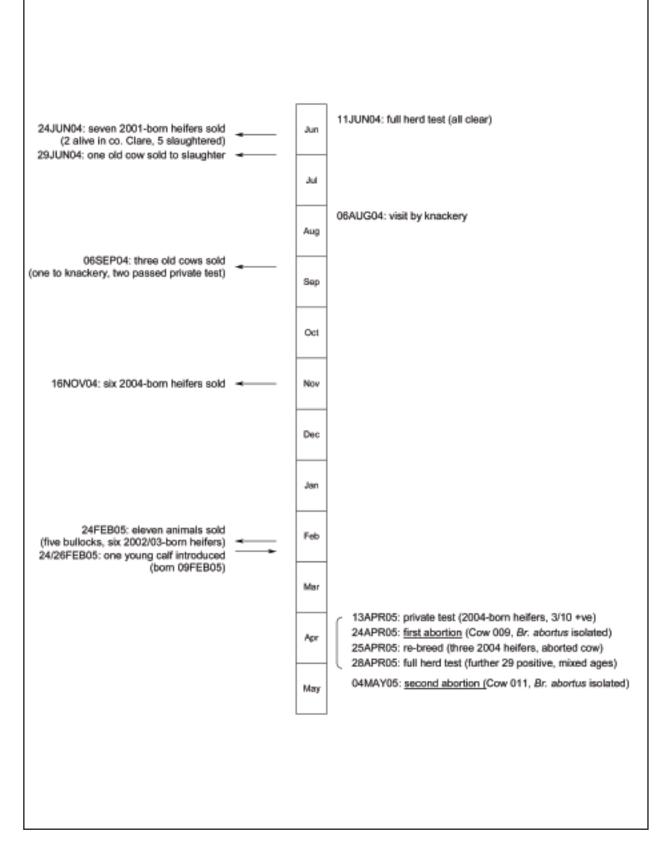


Figure 1. General timeline of events from June 2004 to May 2005

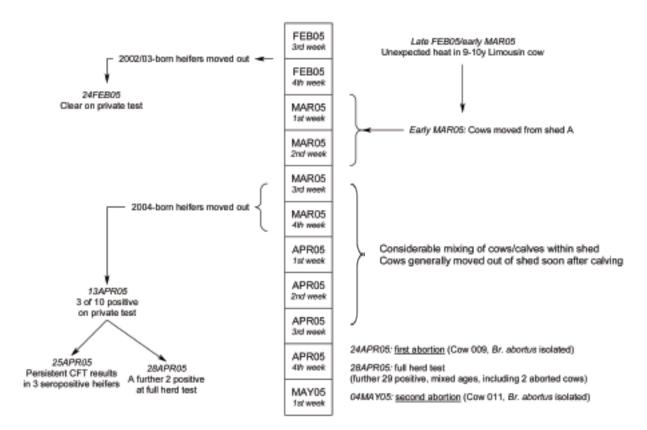


Figure 2. Timeline of events in shed C, during the 3 months from late February 2005

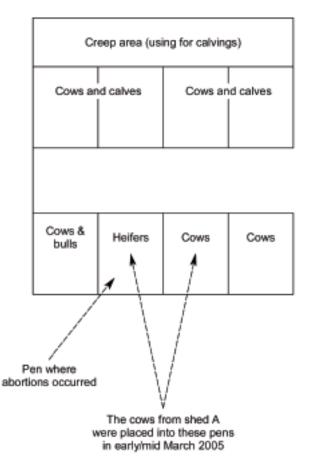


Figure 3. Layout of Shed C during March 2005

## 4. Within-herd transmission

Based on the information available, within-herd transmission on this farm probably did not occur prior to 24 February 2005. There was no evidence of clinical disease in animals at any stage until late April 2005, even though there were pregnant cows in both sheds A and C throughout the winter period. As a consequence of key management events (the close confinement of cattle during winter, pregnant cattle in sheds A and C throughout this period), there was ample opportunity for within-herd transmission and disease expression, if any animal had been infectious. Further, eleven animals were sold on 24 February 2005, included 6 2004-born heifers which had been held for some period up until the sale date with older cows in shed C. Each of these heifers was seronegative on a private test. During subsequent trace-forward investigations, there was no evidence of infection in three surviving animals.

This case highlights the potential for rapid and substantial within-herd transmission of infection. In this outbreak, transmission commenced at some point between 24 February and late March 2005. By late April 2005, 33 of 62 (53.2%) eligible animals (females greater than one year of age, breeding bulls) were seropositive to brucellosis, including 5 of 14 (35.7%) 2004-born heifers, 27 of 46 (58.7%) cows and 1 of 2 (50%) bulls. Each of these animals had been held in shed C for some weeks during March 2005. Further, although the ten 2004-born heifers were moved from shed C to grazing in late March 2005, infection was active in this group at this time with 5 of these animals subsequently testing positive (3 on 13 April 2005, a further 2 on 28 April 2005).

Based on the serological and clinical information, most animals became infected following substantial exposure to a common source of *Br. abortus* during March 2005. The results from the ten 2004-born heifers (13 and 28 April 2005) and from the whole herd (28 April 2005) are each suggestive of recent infection. Because there was rapid seroconversion of large numbers of animals, it is most-likely that the herd was exposed to a substantial quantity of infectious material, probably an aborting cow or aborted foetus, during this time. For this reason, this is probably a case of second generation infection (the first generation of infection involving a single animal which subsequently became highly infectious during March 2005). If this had been first generation infection (for example, infection first introduced onto the farm in March 2005), it is most probable that only a small quantity of infectious material was involved. In such situations, infection may not have become widespread until the infectious load had subsequently increased (for example, following the abortions on 24 April and 4 May 2005).

### 5. The source of infection

After considering all available information, infection was probably introduced to shed C by animals from shed A in early March 2005. This movement immediately precedes the likely start of within-herd transmission within shed C. It also precedes – by approximately one week – the out-movement of the 10 2004-born heifers that were subsequently seropositive on 13 April 2005. Of these 10 older cows, there is only one with any suspicion of earlier infection. This was a 9-10 year old Limousin cow (ID unknown) which was noted to have been bulling at some stage prior to this move (possibly late February or early March 2005), even though the herdowner thought at the time that she was late pregnant. The previous calving date of this cow is unknown. This animal had grazed throughout the farm (with no records of her exact location at any point in time) during the previous summer and autumn.

A range of potential sources of infection have been investigated, as follows:

- Introduction of infected cattle. There was only one introduction onto the farm, in February 2005. This calf had been bought locally, from a herd with no previous or subsequent evidence of infection.
- Other animals. Apart from cattle, other domestic animals on the farm included two donkeys and two dogs. Serological testing was conducted on each of these animals; the donkeys were seronegative, one of the two dogs had a MSAT of 4/80.
- Residual infection. This herd had not been restricted for brucellosis in the previous 15 years.
- Local area spread. Following comprehensive testing, infection was detected in a single animal in a contiguous herd (see previous). Within the locality, brucellosis had last been detected approximately 12 km away, in September 2004. No connection between the two outbreaks has been identified.
- Associated herds. Information was obtained on all herds that had been restricted due to brucellosis in County Clare, and the
  neighbouring counties (Galway, Limerick, Tipperary and Kerry) during the previous 12 month. In particular, data were sought on
  the date and location of each restriction, and disease severity (and likely infection load) in each of these herds. No associations
  were identified.
- Mechanical spread. A range of mechanisms were investigated, involving both people and machinery. These related to the movements of the herdowner and of local veterinarians, a visit to the farm by the local knackery on 6 August 2004, the delivery of feed by a commercial operator, the activities of the local hunt and shooting club, and movements relating to a small commercial quarry that operates on the farm. The location and source of local streams (including one adjacent to shed A) were also investigated. In all cases, no plausible links with introduction of infection were identified. There had been no farm visits by contractors (silage makers, slurry spreaders) during the 12 months prior to the outbreak.
- *Malicious introduction*. We could find no evidence of malicious introduction in this case. The likely index case would have grazed fields adjacent to a regional road prior to winter housing in 2004.

## 6. Conclusion

A range of epidemiological methods, with laboratory support, were used during this investigation. Unfortunately, the source of infection could not be determined. Following introduction, there was rapid within-herd transmission of infection, facilitated by a range of factors, including winter housing (close contact) of cattle and the ongoing mixing of animals (both during grazing and at housing) throughout the year. Disease containment, including the near-absence of contiguous spread, was greatly facilitated by the recent construction of shed C, where most of the animals had been held during winter 2004-05.

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# Descriptive epidemiological findings for eleven cases of Bovine Spongiform Encephalopathy diagnosed in animals born after enhanced controls were introduced in Ireland

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## Abstract

Additional Bovine Spongiform Encephalopathy (BSE) controls were introduced in Ireland in 1996 and 1997. Although highly effective, a small number of cases of BSE have been confirmed in animals born after 1997. This study describes findings at the animal and herd level for eleven of the sixteen cases confirmed to date, the herd of interest being the herd in which the animal was most likely to have been exposed (herd in which the animal spent its first year of life).

In many respects the cases and herds did not differ significantly from those affected before the introduction of additional controls (1995 and earlier). Cases were located in four of the five regions of the country and were not concentrated in previously high incidence areas. Cases were not associated with one particular feed supplier. No evidence of non-compliance with the additional controls could be detected though an association between one case and illegal dumping of specified risk material from adult bovine animals was found. The source of the infectious agent in the herds of putative exposure remains uncertain requiring further investigation of the potential risk factors suggested by this exploratory study.

## 1. Introduction

The first case of Bovine Spongiform Encephalopathy (BSE) was diagnosed in Ireland in 1989. A ban on the feeding of meat and bone meal to ruminant animals was imposed in 1990. In response to emerging scientific information and the identification of a link between BSE and a novel disease of humans (variant Creutzfeldt-Jakob disease), additional controls on the production, storage ands utilisation of mammalian meat and bone meal were introduced in 1996. Although clearly effective at protecting the majority of the cattle population, a small number of cases have been diagnosed in animals born after this and a number of other measures were introduced (so called born after the real ban or BARB cases). These cases fit the pattern of the third, epidemiological-distinct series of cases described by Wilesmith (2002). This paper describes the principal epidemiological features of eleven cases of BSE diagnosed in bovine animals born after 1997. The objective of this study was to generate hypotheses about the potential causes of these cases, which could then form the basis of a later detailed study on causation.

### 2. Materials and methods

Information for this study was collected at the case animal level and at the herd level, the herd of interest being the herd in which the case animal had spent its first year of life as it was at this location that the animal was most likely to have been infected (putative exposure or PE herds). The study involved eleven case animals and ten PE herds (one herd had produced two cases from the same birth cohort). Each herd was visited by specially trained Veterinary Inspectors from the local District Veterinary Office who examined the premises and collected information regarding the case animal, the dam of the case animal, herd management, feeding history and any veterinary medicinal products used on the holding (Table 1). When available records were used (farm, feed supplier or Department of Agriculture (DAF), however, in some instances records describing events which had happened on average 5-6 years earlier were not available and Veterinary Inspectors were forced to rely on herd owner testimony. In one instance, the herd owner was deceased making it difficult to collect any information about feeding history.

### Table 1. An overview of factors considered during the investigation on each case farm

## <u>1. Identification</u>

Examination of CMMS (pattern of registrations, replacement tags, twinning rates), herd file, herd register, animal passport, pedigree records

Visual inspection of carcase, including examination of dentition Forensic analysis of tags, DNA analysis of offspring

### 2. Ingestion

### 2.1 SRM

Detailed spatio-temporal investigation of case (information from area aid maps; location[s] of case during first year of life; schematic map of farm[s]; spatial relationship with any local sources of SRM, including packs of hounds, knackeries, meat plants, rendering plants) On-farm examination (looking for evidence of bones and unusual contiguous activity; illegal disposal of fallen stock; spread of factory waste, knackery waste or waste from rendering plant onto grazing land)

### 2.2 Products containing SRM

Reliant on feed records (supplied herd owner), and extensive sampling for evidence of bone material

a. Meat and bone meal (MBM): gaps in feeding records; detailed feeding history (use of MBM at any stage, source, what about sick animals?, did farmer give any supplements to help recovery?); extensive on-farm sampling (feed, feed residue from buildings)

*b. Feedstuffs to which MBM could have been added:* (in Ireland since 1996, only legally added to pet food and horse supplements) presence of dogs or horses on farm during period of interest, products fed, amounts purchased and storage arrangements

c. Feedstuffs inadvertently cross contaminated with MBM: (seeking a connection between feed stocks manufactured in 1998 and pre-1997 feed stocks or MBM) detailed understanding of normal feeding practices for stock throughout their first year of life; investigation of feed purchase records during year of interest (and one year either side); source(s) of feed; investigation of relevant supplier sale records during years of interest (changes in patterns; gaps; detailed information on feed including name, place purchased, manufacturer, batch numbers)

## 2.3 Atypical BSE

Detailed investigation of clinical history; further laboratory testing (when available); trace, slaughter and test all progeny

### 2.4 Maternal

Detailed investigation of dam (fate; BSE testing results, if slaughtered; clinical history, if died)

### 2.5 Horizontal

Presence of other confirmed cases of BSE on the farm (potential for direct and/or indirect contact between cases); presence of other deaths from CNS disease; linkages with other BSE cases (for example, slurry/manure spread from other farms)

### 2.6 latrogenic (injection or ingestion)

Detailed investigation of farm medicine records (compilation of products, including trade name if available, given or likely to have been given to case in first year of life)

## 3. Results

### 3.1 Animal level

The case animals were born during 1998 (4 cases), 1999 (5), 2000 (1) and 2001 (1). The average age at diagnosis was 62 months (range 43-75). Four of the cases were detected by clinical surveillance (reporting of suspect animals usually by private veterinary practitioners to the competent authority, four were detected during the testing of fallen stock (animals which die or are euthanised on farm), two were detected during routine testing of herds in which BSE has already been confirmed (Ireland has operated a full herd depopulation

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programme for BSE since 1996), the remaining case was detected during the testing of animals over 30 months of age submitted for slaughter. Ten of the case animals were cows the remaining animal was a bull. Eight of the cases occurred in dairy animals (3 Friesians, 1 Friesian Saler cross, 3 Holsteins, 1 Montebeliarde); three occurred in beef crosses of the type commonly used in suckler herds in Ireland (2 Aberdeen Angus, 1 Simmental). Four of the cases were pedigree animals.

All but one of the animals, showed at least some clinical signs prior to death. Weight loss was recorded for 6 case animals. Four animals exhibited hypersensitivity, four demonstrated ataxia, one had muscle tremors, one exhibited teeth grinding and six became recumbent before death.

None of the recorded dams of the affected animals had been confirmed positive for BSE or had produced any other progeny, which had been confirmed positive for BSE. One of the registered dams was purchased and retained at the Department of Agriculture's farm for 2 years after confirmation of BSE in her progeny animal. The dam showed no clinical signs of disease and tested negative for BSE at the time of slaughter. Although one dam had been a cohort of a 1995-born case, this animal tested negative for BSE when traced and slaugh-tered (as a cohort) in 2001. Three further dams were test negative at the time of slaughter. One dam died from an unidentified condition 6 months after the birth of the case animal.

## 3.2 Herd level

PE herds were not been confined to one region of the country (Figure 1). Four of the PE herds were located in the northeast region (Table 2), four were located in the northwest and the remainder were located in the southwest. Seven of the PE herds were predominantly dairy enterprises, three of the seven also had beef fattening units and one had a concurrent sheep enterprises. The other three PE herds were solely suckler operations though the herd owners also kept a few hens. All but one of the PE herds are recorded as having had farm dogs fed predominantly on dry dog food; one suckler enterprise had a pack of hounds which were fed on butchers waste. Two PE holdings had packs of hounds on land adjacent to that grazed by the PE herd. The on-farm investigation in one of these PE holdings revealed the operation of illegal knackery (premises where meat from fallen animals is harvested and fed to packs of hounds) with large scale dumping of carcasses in a site adjacent to land where the case animal spent its first grazing season. Evidence of scavenging by wildlife resulting in bones being deposited on the PE holding was noted.

# Table 2. The location of the 10 PE herds and the year of birth for the 11 Irish BSE cases born during 1998 to 2001 that subsequently developed bovine spongiform encephalopathy

Region	County	Number of PE herds	Year of birth of case animals
North east	Cavan	2 <sup>a</sup>	1998, 1998
	Monaghan	1	2001
	Meath	1	1999
North west	Leitrim	1	1999
	Mayo	1	1998
	Sligo	2	1999, 2000
South west	Kerry	1	1999
	Limerick	1	1999

<sup>a</sup> One of the herds in County Cavan was the herd of putative exposure for two cases of BSE

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Figure 1. The location of the 10 PE herds, where 11 Irish BSE cases had been born during 1998 to 2001

Four of the PE herds had reared a previous case of BSE during its first year of life, with the interval between the previous and post 1997 born case varying from 3 to 10 years. PE herds varied in size; the smallest a suckler herd with just 11 animals (farm size 25 acres), the largest (372 animals) a predominantly dairy operation producing some store cattle (farm size 204 acres). As has been reported previously (Sheridan *et al.*, 2005), larger herds were affected more often (6 of the case farms had more than 100 animals) than smaller herds. There was also considerable variation in management style, with some PE herds feeding high levels of concentrates to maximise productivity, others being managed more extensively with by-products of other industries (bread and whey) being fed in two PE herds.

The bulk of rations fed to animals during the first year of life on all PE holdings were proprietary concentrates purchased from local sources. Although no single feed producer was involved in all cases, PE herds in the same region did have feed producers in common. The majority of case animals were born in spring, as are most calves in Ireland. Milk replacer was not used in five of the ten PE herds either because of the availability of whole milk or the herd was a suckling enterprise. In general, animals on PE farms received concentrate feed-ing through their first year of life, no concentrates at grass during their second grazing season, and small amounts of concentrates with silage during their second winter. Some level of home mixing (in most cases, merely the addition of rolled barley to silage) was performed on five of the ten PE holdings.

Extensive sampling for evidence of terrestrial animal bone was carried out on each PE holding. Samples of dust-like material from feed storage areas were taken in conjunction with routine feed samples. Evidence of terrestrial land animal bone was found on five of the ten PE holdings, as follows:

- On two farms, feed samples taken from a loft used to store feed above the milking parlour tested positive for 1-2 pieces of terrestrial land animal bone and for fish bone.
- On one farm, significant quantities of terrestrial land animal bone were found inside an old silo, which had not been used since 1990.
- On one farm, terrestrial land animal bone was found in old material scraped from the floor of the calf shed. This farm is no longer in operation.
- On one farm, terrestrial land animal bone and fishbone was found in an old silo and in part of a feed system, which had been constructed in 1997.

## 4. Discussion

Both the clinical signs and age at onset are consistent with findings reported by Wilesmith (1998), suggesting that the cases are caused by the strain of agent which has been responsible for all UK cases (so called classical BSE). Further laboratory analysis would be required to verify this finding.

There is no evidence that maternal transmission was responsible for any of the eleven cases reported in this study. However, caution needs to be exercised in interpreting the results as identification of the dam relies on herd owner registrations. In two of the affected farms, herd owner records were shown by DNA testing to be unreliable. In many cases, DNA comparison between dam and case animal was not possible as the dam was already dead by the time the case was identified.

It was not possible to conduct a statistical investigation of the temporal and spatial pattern of PE herds, due to the small number of affected herds. However, there were more PE herds in the north of the country then in the south. Within regions there was some evidence of spatial and temporal clustering which may suggest a common source of infection. The confirmation of four BARB cases reared during their first year of life in the north-west was of particular interest because this was an area of relatively low BSE incidence when compared to other areas such as the north-east. This finding suggests that BARB cases are related to a risk introduced after the implementation of additional controls and not to residual risk from the past, e.g. traces of old feed on farm.

The higher number of dairy farms (compared to suckler farms) affected by BSE in this study is consistent with previous findings for pre BARB cases (Sheridan *et al.*, 2005). Only two BARB cases could be linked with a sheep enterprise and none of the affected holdings had an association with pig or poultry operations, suggesting that contact with scrapie infected sheep or inadvertent access to a farm animal feeding stuffs containing MBM cannot explain all BARB cases. The variation in size and management practises on affected holdings is consistent with pre BARB case of BSE. With the exception of one PE holding, where evidence of direct contact by the case animal with untreated specified risk material (SRM) was found there was no evidence of direct contact with either SRM or MBM.

In Ireland, the majority (>99%) of PE herds during the BSE epidemic have experienced a single case of BSE. In contrast, among the farms with post 1997-born cases, multiple cases have occurred on 40% of PE herds (4 herds) including three with an earlier case of BSE and one where two (post 1997-born) cases were identified within the same cohort. This finding may be a statistical anomaly, given the small sample size. Alternatively, they might provide some evidence of herd level risk factors associated with disease. There is some support for this hypothesis from the UK epidemic, where 80% of cases have occurred on 20% of farms (Hagenaars *et al.*, 2000). Given the time interval between the birth of case animals on these 4 PE farms, however, infection of both cases from the same source (e.g. feed batch) seems unlikely.

Milk replacer has been suggested as a possible cause of BSE because potentially it could contain tallow of bovine origin. Though pure tallow has not been shown to pose a risk with regard to BSE, there is concern that certain types of tallow can contain significant levels of proteinaceous material. However as five of the PE herds had never used milk replacer, contamination of milk replacer is not an explanation for each of the Irish BARB cases.

The findings in relation to terrestrial animal bone are interesting, but must be interpreted with caution. This study reports sampling results from holdings which reared a post 1997 born BSE case, but not from unaffected holdings. Given that there was no observable difference in feed management or hygiene on these PE as compared to many Irish farms, the residual presence of terrestrial animal bone may be widespread. Traces of terrestrial animal and fish bone were found in an old silo on one of the index farms; however, because the case animal had been present on the farm for only  $1^{1}/_{2}$  years prior to the onset of clinical disease, this finding could not have been associated with disease. Four of the five positive samples related to trace amounts of terrestrial bone material (approximately 1-2 spicules). Although the infective dose of infected brain material is very low, the infective dose for MBM manufactured from infected animals is not known. In two of the five cases, the feed stored in the contaminated area was dairy ration for milking cows, which is unlikely to be consumed by animals in either their first or second year of life.

In summary, the epidemiological characteristics of BARB cases and PE herds match those of pre BARB cases suggesting that the route of transmission is the same. All cases cannot be explained by maternal transmission, contact with scrapie infected sheep or access to pig and poultry rations containing MBM. Further, with one exception these cases cannot be explained by direct access to SRM or MBM. Contamination of milk replacer cannot explain all cases. Though the possibility of inadvertent access to dry dog food containing MBM cannot be ruled out, this is an unlikely source of disease given the very low probability of exposure. Also, under European Union Regulations MBM used in pet-food must be treated at 133°C, 3 bar pressure for 20 minutes, producing at least a three log reduction in infectivity. Likewise though the possibility of contact with pre-1996 infective feed material cannot be ruled out, the probability that this is responsible for all cases is low.

This study met its principal objective suggesting that BARB cases in Ireland may be caused by feed borne transmission, the precise mechanism of which requires further study. No evidence could be found of failure to comply with the additional controls introduced in 1996 and 1997. Beyond DAF issuing advice to all farmers to have feed storage areas cleaned, no areas where the existing controls can be improved were identified. The study highlighted the very valuable resources of the DVO Veterinary Inspectorate who can carry out high quality epidemiological investigations. Attention to visual examination and the value of walking the lands and talking to neighbours were highlighted by the discovery of an illegal dump of animal by-products adjacent to a PE farm.

## Acknowledgements

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# The economic impact of Johne's disease in an Irish dairy herd: A case study

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### Abstract

An epidemiological investigation, examining the economic impact of Johne's disease in an Irish dairy herd, concluded that infection was introduced into the herd in 1993 with the importation of 20 Dutch heifers. The practice of feeding pooled colostrum and milk was considered to have disseminated *Mycobacterium avium* subspecies *paratuberculosis* (MAP) widely throughout the herd. Farm performance declined substantially between 1993 and 2003, as a result of reduced milk yields, increased culling and reduced cull cow values. This negatively impacted on the profit margin per litre milk sold and per cow. The performance relative to a group of 25 to 30 peers also deteriorated over the study period. Farm performance was superior to that of its peer group until the late 1990s, but was markedly worse by 2002. Profit margin per cow had been €272 greater than, but fell to €230 less than, the group median in 2002. Similarly, when compared to the group median, average milk yield per cow was 814 (14.7%) litres above, but fell to 778 (13.9%) litres below in 2002. Economic recovery commenced in 2003 as a result of the application of control measures that were applied from 2002 onwards.

## 1. Introduction

Paratuberculosis (Johne's disease) is a chronic granulomatous enteritis of ruminants, including cattle. It is caused by *Mycobacterium avium* subspecies *paratuberculosis* (MAP). The disease is characterised by persistent diarrhoea, weight loss and protein losing enteropathy. Most infected cattle excrete the bacteria in faeces months to years before clinical signs of infection develop (Sweeney, 1992). Eradication of Johne's disease requires the removal of all sources of infection, the dominant source of infection being the presence of sub-clinically infected animals (Whittington and Sergeant, 2001). Control programmes in dairy herds involve hygienic measures to prevent the establishment of infection in young calves, and the systematic culling of infected animals.

Johne's disease can cause significant economic loss in affected herds. Losses are associated with reduced milk yield, lower reproductive efficiency, premature culling and decreased cull cow values. Johne's disease has been a scheduled and notifiable disease in the Republic of Ireland since 1955. It was uncommon prior to the mid 1990's, with only 92 cases diagnosed between 1932 and 1992; these cases were primarily in imported animals (Dept. of Agriculture and Food records). In 1992, the Single European market was introduced, facilitating the free movement of goods and services within the EU and thereby increasing the opportunity for the importation of cattle from continental Europe. The Single Market removed the national pre-import test and certification requirements for Johne's disease and also the requirement for imported livestock to be placed in quarantine for up to six months after arriving in Ireland. During quarantine, imported animals had been subjected to additional Johne's disease test. Between 1992 and May 2004, approximately 85,000 cattle were imported from continental Europe the bulk of which were potential breeding animals. Of these, 8,223 came from the Netherlands, 6,832 from Denmark and 29,105 from France (Central Statistics Office, personal communication). In the years between 1995 and 2002, the Dept. of Agriculture and Food received notification of 232 Johne's disease infected cattle in 106 herds. In 1997, a serological survey, using the absorbed ELISA test, of 224 imported animals in 36 herds, revealed that 36% of the herds involved had at least one positive animal (O'Doherty *et al.*, 2002). Using the same test in a random sample of 143 herds in three counties, more than 30% of herds had one or more reactors (J. Egan, personal communication). The indications are that the prevalence of Johne's disease in Ireland has increased since the introduction of the Single European market.

This paper describes the impact of an outbreak of Johne's disease on performance in a dairy herd in Co. Tipperary, Ireland.

### 2. History

The case farm is family-owned and managed by the owner with some outside assistance. All cows were Holstein Friesians prior to the introduction of Montbéliarde cattle in 1998. Replacement dairy cattle were purchased from the Netherlands in 1993 (Figure 1). The aim, for many years, has been to have cows calving at a 365-day interval. Artificial insemination (AI) is still used in the earlier stages of the breeding season, while natural service is used subsequently to serve any cows not in calf to AI. Breeding cattle are routinely vaccinated biannually for leptospirosis and cattle are vaccinated against black disease two to three times per year. During 1993 to 2004, the average herd size was 81, varying from a low of 69 in 1996 to 90 in 2001. The first clinical signs associated with Johne's disease were observed on the case farm in 1995, and the first laboratory diagnosis of the disease was made in April 2000. In retrospect, an addition-

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al 11 cows, four of which were the original Dutch imports and others progeny of these, were identified as potentially Johne's infected between 1995 and 2000, based on clinical signs of ill thrift combined with persistent diarrhoea. A widespread herd Johne's disease problem was diagnosed in 2002. Observed clinical signs included persistent diarrhoea, weight loss, illthrift and depressed milk yield. The farmer observed "bottle jaw" or intra-mandibular oedema in the later stages of the disease. He said that cows remained bright and alert up until the end stages of the disease. He also noted in retrospect that affected cows appeared less fertile.

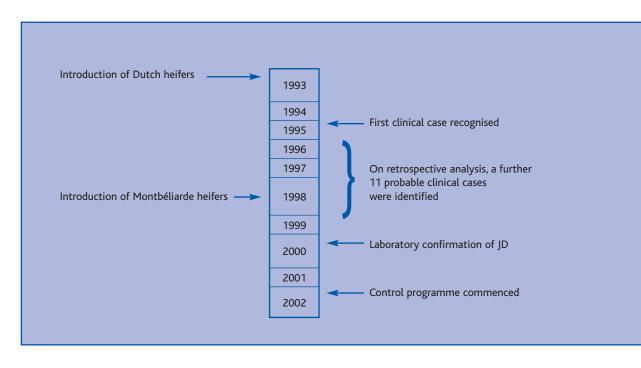


Figure 1. Time line from introduction of infection to introduction of control measures

## 3. On-farm investigation

This case was brought to the attention of the national Department of Agriculture and Food (DAF) in late February 2002. The farmer agreed to participate in a pilot Johne's disease control programme under the auspices of DAF. A detailed epidemiological examination was conducted to determine how Johne's disease had entered and spread within the herd. This investigation concluded that Johne's disease was introduced through the purchase of a cohort of 20 heifers from the Netherlands in 1993. Up to four of these imported animals went on to develop clinical signs consistent with a diagnosis of Johne's disease, but this was not confirmed by laboratory diagnosis. At least five of the progeny of these imported animals were subsequently diagnosed with Johne's disease. The farmer had not previously purchased cattle from outside Ireland. There was no evidence to suggest that Johne's disease was in the herd prior to the introduction of the Dutch cattle in 1993.

The practice of feeding pooled milk and colostrum was considered to have facilitated the spread of infection within the herd. The farmer routinely fed pooled colostrum to calves up to seven weeks of age. Examination of farm records revealed clusters of infection among calves, which were known to have been consuming milk when cows, which subsequently exhibited clinical signs consistent with Johne's disease, were providing milk into the calf milk supply.

## 4. Farm productivity and profitability

The case farm formed part of a wider Teagasc (Irish Agriculture and Food Development Authority) DairyMIS group. This DairyMIS group consisted of 25 to 30 herds of a similar size in the Munster region, which recorded various production and economic data for Teagasc.

### Average milk yield

In 1994, a large number of heifers entered the herd, which reduced the milk yield of the herd in that year. Between 1995 and 1998, the average milk yield for the case herd was in the upper quartile of the DairyMIS group, and was just short of the 90<sup>th</sup> percentile in 1995 and 1997. By 1999, average milk yield had decreased to that of the group median and it continued to fall to the 10<sup>th</sup> percentile for the group in 2002 (Figure 2). For example in 1995 and 1997 respectively, average yield was 714 litres (13.6%) and 814 litres (14.7%) litres per cow greater than that of the group median. A decline in herd yield in the case farm relative to its peers commenced in 1997 and continued until 2002 when the average yield was 778 litres (13.9%) litres less than that of the group median, and indeed was almost equivalent to the 10<sup>th</sup> percentile. In the case herd, there was a difference of 1,528 litres (24.0%) in the average milk yield between the years with the best and worse milk yields (1997 and 2002). Milk yield in 2003 and 2004 respectively were 5,636 litres and 5,418 litres.

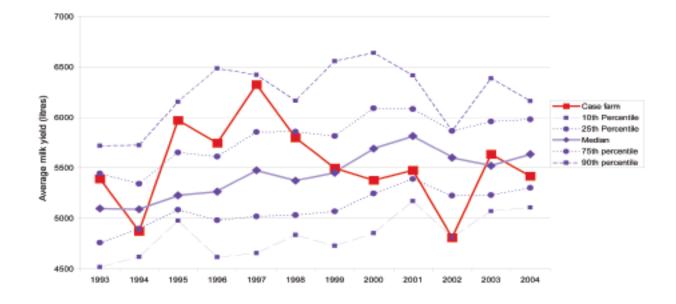


Figure 2. Milk yield during 1993 to 2004: case farm, in comparison with peers in the DairyMIS group

### Profit margin per cow

The profit margin per cow was calculated by adding the value of milk sold plus the value of milk fed to calves, subtracting the value of concentrate fed and fertiliser spread and dividing this by the average number of cows in the herd. Until 1999, the profit margin per cow exceeded that the median of the peer group (Figure 3), indeed profit margins per cow were in the upper quartile for much of the 1990's and was on average  $\in$  155 greater than that of its peers. In 1995 and 1996, respectively, the profit margin per cow was  $\in$  264 and  $\in$  272 greater than that of the medians for the years involved. Equivalent figures of profit margin per herd were  $\in$  22,968 and  $\in$  18,768, respectively. However, there was a decline in the profit margin on the case herd starting in 1997; in 1999 the profit margin per cow was equivalent to the group median, and in the lowest group quartile from 2000 until 2003. Indeed from 1999 until 2003, the margin per cow in the case herd was on average  $\in$  130 less than that of its peers. The profit margin per cow was  $\in$ 168 ( $\in$  12,768 for the herd) and  $\in$ 253 ( $\in$  19,734) less than the group median in 2001 and 2002, respectively. This represents a cumulative reduction in margin per cow of  $\in$ 285 over the course of the study period. Profit margin per cow increased in 2003 and 2004, reversing the decline in previous years.

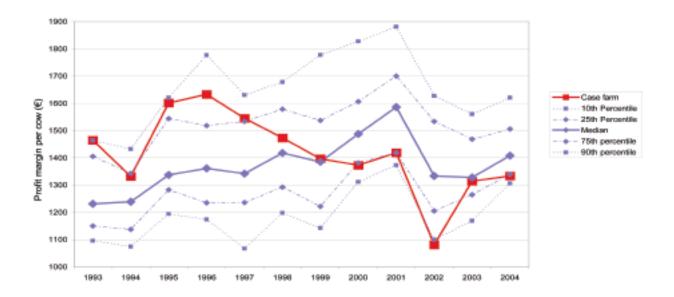


Figure 3. Profit margin per cow 1993-2004: case farm, in comparison with peers in the DairyMIS group

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## Involuntary culling

Involuntary culling consisted of all cow disposals, apart from disposals due to cows being surplus to requirements or old age. Prior to 1997 involuntary culling in the case herd was less than that of the group median (Figure 4). The involuntary culling rate in 1997 and 1998 was slightly greater than the median for the group. It diminished between 1999 and 2001, where it was equivalent to 25<sup>th</sup> percentile, but peaked in 2002 and 2004. Involuntary culling was in excess of the 90<sup>th</sup> percentile for the group in 2002.

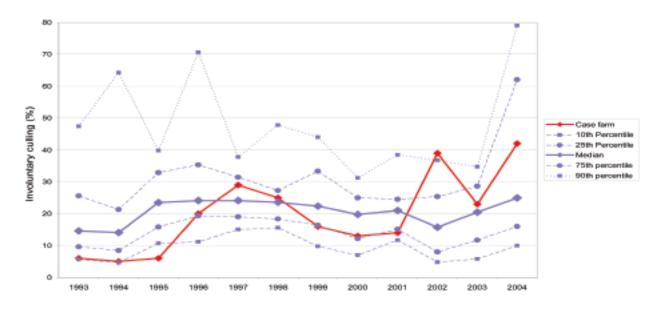


Figure 4. Involuntary culling 1993-2004: case farm, in comparison with peers in the DairyMIS group

### Culling due to infertility

A closer examination of the relative causes of culling in the herd revealed that there was a marked increase in the proportion of involuntary culling due to infertility between 1994 and 2000 (Figure 5). Between 1996 and 2000, infertility accounted for between 40% and 80% of involuntary culling in the case herd, and was in the upper quartile for the group in 1996, 1998 and 2000. Between 1996 and 2000, the group median for the proportion of cows culled due to infertility was 34-50%. From 2002 infertility declined as the main cause of involuntary culling.

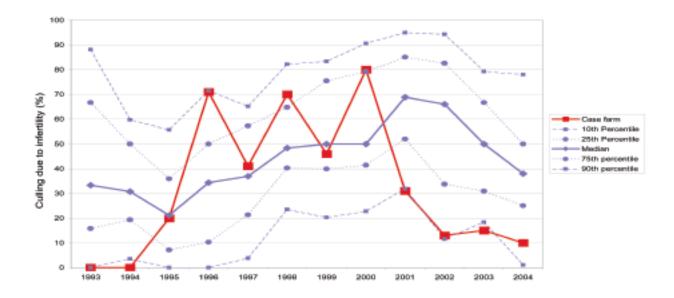


Figure 5. Percentage culling due to infertility during 1993 to 2004 on the case farm, in comparison with peers within the DiaryMIS group

## 5. Discussion

The farmer responsible for the herd under investigation here was seeking to improve herd genetic merit, but had concerns about the risk of brucellosis associated with native sourced cattle and hence imported Dutch heifers. At that time he was unaware of Johne's disease, and therefore did not consider it as a relevant animal health risk. Ignorance thus played a major role in the introduction and dissemination of Johne's disease in this herd. Failure to maintain a closed herd (i.e. introducing cattle from outside) has been shown to result in a 5% reduction in net margin (van Schaik *et al.*, 1998). A US study found that almost half of US dairy managers had limited knowledge of Johne's disease, which has hampered the effectiveness of control programmes (Wells and Wagner 2000).

It is probable that Johne's disease was introduced into the case herd with the Dutch heifers. There are several reasons to support this view. There was no history of Johne's disease in the herd prior to their introduction. Further, the purchase of infected cattle is recognised as a significant method of introducing Johne's disease into herds (Cetinkaya *et al.*, 1997; Wells and Wagner, 2000). At the time up to 55% of Dutch dairy herds had one or more animals serologically positive for Johne's disease (Mushens *et al.*, 2000) and the imported animals would have originated in between six and nine herds. Finally, up to four of the animals imported went on to develop clinical signs consistent with Johne's disease as did a number of their progeny.

Following on from Johne's disease diagnosis in 2002 the farmer was anxious to implement a control programme in an effort to reverse the significant economic losses experienced in the previous few years. He had been a successful and profitable farmer prior to the emergence of Johne's disease in his herd. Reduced milk yield, lower feed conversion efficiency, increased involuntary culling, higher replacement rates, decreased fertility, increased mortality and reduced cull cows values are all synonymous with Johne's disease (Ott et al., 1999). DairyMIS data revealed that average herd yields, milk protein content, margin per 1000 litres of milk produced, margin per cow and culling rates were superior or equal to those of his peers until the late 1990's. However, from the mid 1990's there was a steady decline in farm performance until 2002 when the Johne's disease control programme was introduced on the farm. There was a 24% difference between the best (1997) and the worst (2002) annual average milk yield over the course of the study period. It was not possible to determine how much of this reduction in milk yield was directly attributable to Johne's disease. Data from North America have documented reductions of 19.5% and 15%, respectively, among cows clinically and sub-clinically infected with Johne's disease (Chi et al., 2002). Lower yields during 2002, in all herds in the DairyMIS group, may have been weather-related; nonetheless, the yields in the case herd were 13.9% less than that of the group median. The extent of the reduction in milk yield has been correlated to the prevalence of Johne's disease infection within herds (Ott et al., 1999). The reduction in milk yield is more pronounced as cows advance in age as infection becomes more advanced (Johnson et al., 2001). These factors may have contributed to the dramatic reduction in yields in 2002, when the problem was finally diagnosed and a considerable number of clinical cases became apparent. In the case herd, there was both an absolute and relative drop in average milk yield. Milk yield was in excess of the group median in 2003, but declined closer to the 25<sup>th</sup> percentile in 2004 when in order to re-build cow numbers proportionately more of the herd comprised home-bred 1<sup>st</sup> lactation animals than usual.

In the USA it has been reported that Johne's positive herds experience an economic loss of \$100 per cow, compared to Johne's negative herds (Ott *et al.*, 1999). The same study also reported that herds with 10% or more of their cull cows having clinical signs consistent with Johne's disease suffered economic losses in excess of \$200 per cow.

Reports published from the UK (Esslemont and Kossaibati, 1997) and Australia (Stevenson and Lean, 1998) describe average involuntary culling rates of 22% and 24%, respectively. The median involuntary culling rate of the DairyMIS group was between 16% and 24%. In the case herd, the involuntary culling rate did not markedly exceed the peer group median until 2002 when the control programme was instigated. This finding was somewhat unexpected as it was suspected that Johne's disease would have increased the level of involuntary culling in the herd. However, it is possible that some of the cows culled voluntarily as surplus to requirements or where the farmer's perception was that the cow was infertile may have been related to Johne's disease.

In the above mentioned UK and Australian studies, infertility accounted for 36.5% and 32% of cows culled respectively, which is substantially less than the range described in the case herd of 50 to 80% between 1996 and 2001. While the group median for culling due to infertility was greater than that described in the UK and Australian studies, this may be a consequence of the grass-based production system and seasonal calving pattern in Ireland, where many dairy herds cease to attempt to impregnate cows once they will exceed a 365-day calving interval. The culling due to infertility in the case herd was in upper quartile for much of the period between 1996 and 2000. Although culling due to infertility was increasing in the peer herds, the group median was still less than that of the case herd. This high level of culling due to infertility in the study herd probably prevented a marked deterioration in the herd calving index. Johne's disease may have been partly responsible for the failure of so many cows to attain the ideal 365-day calving interval required on the case farm and therefore their consequential cull for 'infertility'. The marked increase in the culling rate in conjunction with a marked reduction in the proportion of cows culled for infertility in 2002, 2003 and 2004 came about due to the culling Johne's infected animals as part of the control programme. The economic loss on the case farm was further compounded by the farmer's difficulty in finishing cull cows for slaughter in the latter years of the study period. It was not possible to quantify this loss but a US study estimated that there was a 25% reduction in cull cow values in herds infected with Johnes disease (Chi *et al.*, 2002). The Johne's status of the peer herds in the DairyMIS group is undetermined and it is possible that in making the comparison to these herds that the economic impact of Johne's disease in the case herd is underestimated. While this case study relates to only one herd, which may not be representative of the 'average' Irish farm, the fact remains that substantial economic loss occurred consequent to the entry of Johne's disease into the herd. These losses have been reversed with the implementation of control measures, and the farmer involved is confident that he can farm his way out of the problem and return to profitability. The study involving this herd continues.

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