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

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Abstract

In Ireland, the herd prevalence of bovine tuberculosis has remained stable for several decades, and in common with several other countries, progress towards eradication has stalled. There is evidence in support of the potential role of infected badgers (*Meles meles*, a protected species) in bovine tuberculosis in Ireland and Britain. However, this evidence on its own has not been sufficient to prove disease causation. Field trials are likely to offer the best opportunity to define this role. Building on the earlier East Offaly project, our objectives were to assess the impact of badger removal on the control of tuberculosis in cattle herds in Ireland.

The study was conducted from September 1997 to August 2002 in matched removal and reference areas (average area of 245.1 km^2) in four counties: Cork, Donegal, Kilkenny and Monaghan. Badger removal was intensive and proactive throughout the study period in the removal areas, but reactive (in response to severe tuberculosis outbreaks in cattle) in the reference areas. Removal intensity in the removal and reference areas during the first 2 years of the study averaged 0.57 and 0.07 badgers/km^2/year, respectively.

The outcome of interest was restriction of cattle herds due to confirmed tuberculosis, where tuberculous lesions were detected in one or more animals. Data were analysed using logistic regression (modelling the probability of a confirmed herd restriction) and survival analysis (modelling time to a confirmed herd restriction).

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During the study period, there was a significant difference between the removal and reference areas in all four counties in both the probability of and the time to a confirmed herd restriction due to tuberculosis. In the final year of the study, the odds of a confirmed herd restriction in the removal (as compared to the reference areas) were 0.25 in Cork, 0.04 in Donegal, 0.26 in Kilkenny and 0.43 in Monaghan. Further, the hazard ratios (removal over reference) ranged from 0.4 to 0.04 (a 60–96% decrease in the rate at which herds were becoming the subject of a confirmed restriction).

Keywords: *Mycobacterium bovis*; Tuberculosis; Cattle; Badgers; Ireland; Logistic regression; Cox model

1. Introduction

A national bovine tuberculosis-eradication programme was initiated in Ireland in 1954. Its essential components were the tuberculin testing of all herds, restrictions on animal movement from infected holdings and strategic infection-control measures in areas of high prevalence. Although initial progress was good (leading to a considerable reduction in herd prevalence by the mid 1960s), the programme subsequently stalled. Difficulties with the eradication of tuberculosis in cattle have arisen also in other countries, e.g. New Zealand, Great Britain, Northern Ireland, and more recently, the State of Michigan in the USA.

Badgers were given legally protected status in Ireland in 1976. Over the subsequent 28 years, evidence has been building of the potential role of infected badgers (*Meles meles*) in bovine tuberculosis, including: isolation of *Mycobacterium bovis* in badgers in Switzerland, the UK and Ireland (summarised by Olea-Popelka et al., 2003); recognition that badgers were highly susceptible to *M. bovis* infection (Gormley and Costello, 2003), and that tuberculosis was endemic within the badger population in Ireland (O'Boyle et al., 2003); increasing recognition of the possible role of wildlife as reservoirs for *M. bovis* in the UK (Krebs, 1997) and New Zealand (Coleman, 1988; Cook, 1975; Hickling et al., 1991; Julian, 1981); the identification of identical strains of *M. bovis* in local cattle and badger populations (Costello et al., 1999); and ongoing tuberculosis problems, despite intensive infection-control efforts aimed at early detection and prevention of cattle-to-cattle transmission. However, this information on its own is not sufficient to prove disease causation. In particular, there is little direct evidence in support of a temporal relationship (providing evidence for transmission of *M. bovis* from badgers to cattle). To illustrate, it is possible to have coincident disease (with identical strains) in local badgers and cattle but without badgers being the source of infection. This could occur, for example, if cattle were to infect badgers, and not vice-versa.

Given this context, field trials are likely to offer the best opportunity definitively to determine the role of badgers in bovine tuberculosis. The East Offaly project in Ireland was the first study of this type, comprising a 738-km^2 'project' area (where badgers were removed under license) in county Offaly and a surrounding 1455 km^2 'control' area (Eves, 1999; O'Mairtin et al., 1998a, 1998b). Smaller UK-based trials have been conducted at Thornbury (104 km^2, in Avon), at Steeple Leaze (12 km^2, in Dorset) and at Hartland (about 62 km^2 in North Devon) (Hansard (House of Commons Daily Debates), 2004). Results from the East Offaly project suggest that the badger-removal programme was effective in reducing the risk of (disclosure of) a tuberculin reactor in a herd. Indeed, in the final year of the study, the odds of a disclosure of a reactor in a herd were 14-times higher in the control as compared to the project area (O'Mairtin et al., 1998a).

Our current study seeks to build on the East Offaly project, and to determine the effect of badger removal at a number of sites representing a wider range of farming environments. Further, we seek to address concerns with that project, relating to the design of the project ('removal') and control ('reference') areas, which resulted in ongoing migration of badgers from the control into the project area throughout the study period (Eves, 1999). Additionally, the East Offaly project represented only one type of farming environment in Ireland. Therefore, our objective of the current study was to assess the impact of badger removal on the control of tuberculosis in cattle herds in a wider range of environments in Ireland.

2. Methodology

2.1. General study design

2.1.1. Overview

In 1996, Duchas (the Irish agency responsible for heritage and wildlife) agreed to issue licences for the large-scale removal of badgers for scientific purposes in four areas of Ireland. Subsequently, this study was designed to assess objectively the impact of badger removal on tuberculosis control in Irish cattle herds. The study was conducted between 1 September 1997 and 31 August 2002 (the 'study period') in matched removal and reference 'study areas' in four different geographical regions in Ireland (counties Cork, Donegal, Kilkenny and Monaghan; Fig. 1). In each removal and adjoining buffer area, initially, as many badgers as possible were removed; then badger numbers were kept as low as was feasible throughout the period of the study. In the reference area, badgers were removed during the study period only on and around those farms where major outbreaks of tuberculosis were recorded. Comparison is made with the 5-year 'pre-study period', which concerns 1 September 1992 to 31 August 1997.

2.1.2. Selection of the removal areas

Purposive sampling was used to select the removal areas. Key selection criteria included: apparent disease prevalence (that is, 'problem areas' with historic or recent evidence of higher-than-average apparent disease prevalence); the presence, if possible, of natural geographical boundaries (such as rivers, mountain ranges and sea inlets); and areas considered representative of the diverse Irish landscape. Where natural barriers were absent, 'buffer areas' were created, up to 6 km in width, at the boundary of each selected removal area.

2.1.3. Selection of the reference areas

Purposive sampling methods were used to match each removal area with a defined reference area within the same county. The matching criteria were based on factors known to influence badger density and herd prevalence of tuberculosis, including livestock density, herd size, farm-enterprise type, disease prevalence (based on the number of tuberculin reactors per thousand animal tests, APT) during the 9 years prior to study start, and selected geographic features. The latter criteria, including land use and soil type, are

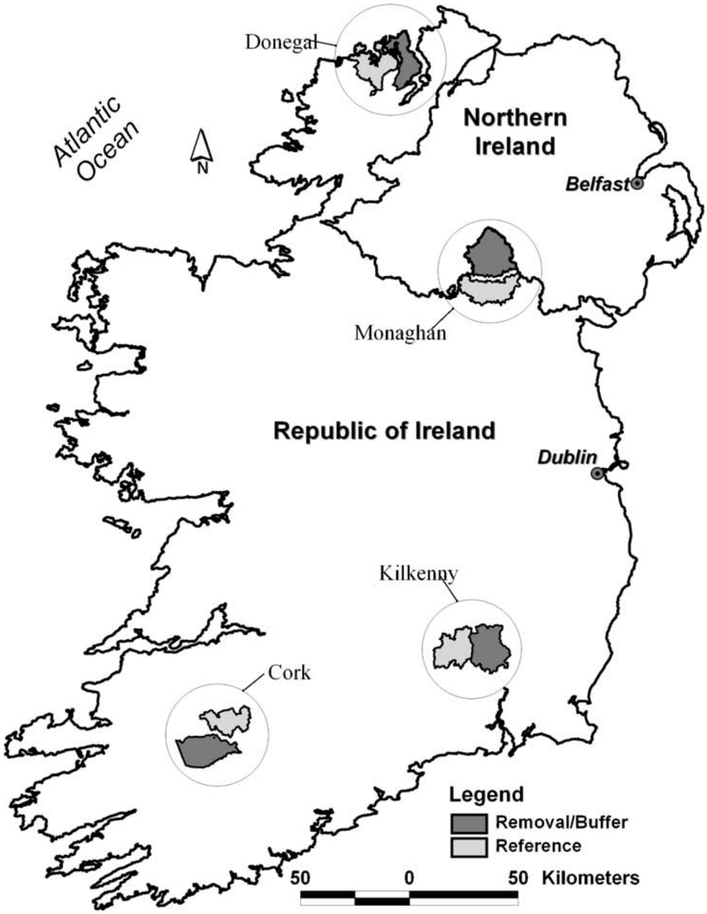


Fig. 1. Location of the matched removal and reference areas in counties Cork, Donegal, Kilkenny and Monaghan in Ireland.

known to influence badger numbers (Hammond et al., 2001). In addition, but only when natural barriers were absent, each reference area was separated from both the removal and (where present) adjoining buffer areas by a distance of at least 3 km, to minimise the effects of badger migration on tuberculosis levels in cattle in these areas.

The matched removal and reference areas in each county were supervised by a single District Veterinary Office (DVO), and managed by a single appropriately trained team throughout the study period.

2.2. The herds of interest

2.2.1. Aspects of the Irish bovine tuberculosis-eradication programme

The testing regimen in the Irish bovine tuberculosis-eradication programme has been described in detail elsewhere (Caffrey, 1994; O'Mairtin et al., 1998a, 1998b). Briefly, herd owners legally are obliged to present their animals for a full herd test each year. At this test, and at a minimum of 60-day intervals when a herd is restricted, the infection status of each animal is assessed by a veterinarian using the single intra-dermal comparative tuberculin test. Following the detection of infection, herds remain restricted until two consecutive negative herd tests are achieved. In accordance with European Community legislation, official Veterinary Inspectors examine all slaughtered animals during abattoir surveillance, either routinely or following a positive herd test.

2.2.2. The study herds

The ‘study herds’ included all herds wholly contained within each removal or reference area. Therefore, herds within a removal area, but with any land fragments elsewhere (either in a reference area, a buffer area, or elsewhere) were not considered in any subsequent analyses. The same was true for herds within a reference area, but with any land fragments elsewhere (either in a removal area, a buffer area, or elsewhere). The locations of herds and herd owners were identified using the digitised Land Parcel Identification Scheme (LPIS) of the Irish Department of Agriculture and Food (DAF).

2.3. Badger removal

2.3.1. The badger-habitat survey

Farm participation in the survey was voluntary. On all participating farms, a comprehensive survey of badger habitat and activity was conducted of all land parcels within each removal, buffer and reference area prior to the start of the study. In these areas, using a team of approximately 30 surveyors, all fields and hedgerows were examined for badger setts. Further, selected parts of the areas were re-surveyed during the study by a badger ecologist. The location of each sett was recorded using a geographical information system.

2.3.2. The programme of badger removal

Details of the badger-removal procedure, including the equipment required, are given in the Badger Manual prepared by the Department of Agriculture, Food and Forestry (DAFF, 1996). Briefly, restraints were placed at active setts for 11 nights and were inspected each morning; occasionally restraints were left in place for a second 11-night period depending on the level of badger activity. Each consecutive 11-night period is considered a ‘removal operation’. Badgers were captured using a multi-strand steel wire which was 143-cm long with a stop at 28 cm. Restraints were laid on badger tracks in a loop secured by an angle iron and wooden support stakes. During the removal period, each removal site was inspected each morning by a trained operative and captured badgers were killed by a member of the project team using a 0.22 calibre rifle (Eves, 1993). All operators involved in this work were trained in the safe use of firearms and the euthanasia of badgers.

Farm participation in the programme of badger removal was also voluntary. Except where permission was refused, a programme of badger removal was carried out under licence on two to three occasions each year on all land fragments within each removal and buffer area. This high frequency of removal was necessary to maximise removal of all resident badgers and any badgers that succeeded in migrating into the area during the study period. With respect to land fragments within each reference area, badger removal was undertaken only following severe outbreaks of tuberculosis in cattle herds (defined as outbreaks where four or more standard tuberculin reactors were disclosed) where the source reasonably could be attributed to badgers. In Ireland, an animal is considered a standard reactor if the bovine reaction in the single intradermal comparative tuberculin test is more than 4 mm greater than the avian reaction, or there is oedema, exudative necrosis, heat and/or pain at the bovine tuberculin injection site and/or swelling of the related prescapular lymph node (DAF, 2003). All outbreaks were investigated by a Veterinary Inspector, and in each case where removal was undertaken, active badger setts were found on or in the vicinity of the farm-in-question and all other sources of infection (residual, purchased and farm-to-farm spread) were considered unlikely. Although removal was generally limited to a single removal operation, removals were repeated if evidence of badger activity was subsequently detected.

2.3.3. The assessment of infection status amongst badgers

A gross *post mortem* investigation was conducted on all euthanased badgers. If gross evidence of tuberculosis was detected, all affected tissues were sent for histopathological examination and for culture. If no evidence of tuberculosis was found, bacteriological culture was conducted on a pool of defined tissues, including lymph nodes, kidney and lung tissue. A badger was considered positive for tuberculosis only if it was positive at histopathological examination and/or culture.

2.4. Data collection

In Ireland, herd-testing data and *post mortem* findings from reactor animals are collected routinely by all local District Veterinary Offices. In addition, data relating to badger removal (location, age, sex, weight and infection status) and breakdown investigations were collected throughout the field trial by project staff either directly or via a participating veterinary laboratory.

2.5. Statistical analyses

2.5.1. Data assembly

The data were assembled into a single database and edited at the Centre for Veterinary Epidemiology and Risk Analysis (University College Dublin). Subsequently, the herd tuberculin-testing records of all study herds were examined to determine periods of permission to trade, trading restrictions due to tuberculosis, and periods without cattle. Each period of restriction then was classified as confirmed (that is, tuberculous lesions were detected in ≥ 1 animal during abattoir surveillance, either routinely or following a positive herd test) or not. Following its first tuberculin test, a herd was considered depopulated during any between-test interval of >2 years (giving missing values for the analysis). For a between-test interval of <2 years, the herd's trading status was known.

To compare the reference and removal areas prior to and during the study, a yearly confirmed restriction status was assigned to each herd in each year (from 1 September to the following 31 August) for 1992/1993 through 2001/2002. The final 5 years (i.e. beginning on 1 September 1997) covered the study period. If the herd entered the year free of a confirmed restriction, then it was considered free of a confirmed restriction for the year if and only if it remained so throughout the year. If the herd entered the year with a confirmed restriction, but became and remained unrestricted during that year, it also was considered unrestricted for that year; otherwise, it was considered to have a confirmed restriction that year. This leads to a confirmed-restriction prevalence. In addition, a survival time for each herd was calculated as the time from 1 September 1992 to a confirmed restriction or to 31 August 2002, whichever came first. In the latter case, the survival time was censored. In cases where no herd test was carried out in the 2 years prior to the end of the study or where a between-test interval is >2 years (see above), the survival time was censored at the time of the last herd test. Additional survival times from the end of a restriction period were calculated for herds with more than one restriction. The survival times were based on time to restriction (under the Irish testing program), not time to infection (which is unknown).

Explanatory variables considered for inclusion in the models were as follows: TR, a factor with two levels denoting the removal (=1) and reference (=0) areas; herd size (time dependent), the number of animals tested at a full herd test; a factor CO with four levels denoting the counties; and a time-dependent factor PH (previous history) with two levels denoting whether the herd had (=1; 0 = no) previously had a confirmed restriction. YEAR was used to denote a factor with 10 levels representing the years 1992/1993, 1993/1994, . . ., 2001/2002. A time-dependent factor PERIOD with two levels corresponding to the pre-study (=0) and study (=1; i.e. from 1 September 1997) periods was used to summarise results.

2.5.2. Descriptive statistics

Descriptive statistics on the number of badgers captured and the proportion deemed to be positive for tuberculosis are presented. Chi-squared was used to test whether there was a significant difference in the proportion of badgers infected in the removal areas compared to the reference areas.

The Kaplan–Meier estimate of the survival function (Collett, 1994) was computed separately for the removal and reference areas within each county, and these were compared using the non-parametric Wilcoxon test.

2.5.3. Statistical models

The study aimed to compare the impact of badger removal at two levels on confirmed restrictions in four different farming environments. Two outcome measures were chosen: (1) yearly confirmed herd-restriction prevalence; (2) survival time to a confirmed herd restriction. Comparisons were made between the removal and reference areas in a county, between study and pre-study years in an area, and between counties. Alpha was 0.05 (2-sided).

2.5.3.1. Logistic model using a generalized estimating equation. A logistic model related the *logit* of the confirmed restriction prevalence to the two treatment areas, four counties, herd size, previous history and year. A generalized estimating equation (GEE) method was used to account for dependence in measures on herds with more than 1 restriction. No dependence is generated otherwise (Allison, 1995, page 223). Estimates using independence and first-order autoregressive-correlation (AR1) structures were compared. Terms were assessed for inclusion in the model on the basis of the generalised score test

(Boos, 1992). Consistent estimates of coefficient standard errors were obtained using the empirical covariance matrix of parameter estimates resulting from the GEE method.

The factor TR was regarded as nested within YEAR, by including terms YEAR and TR × YEAR in the model. These terms further were nested within CO, producing a model with three-way interactions. Interactions involving PH, CO and TR were tested. An annual herd size for each herd was taken as the mean number of animals tested at all full herd tests during the year, or the number of animals tested at the previous such test if all tests during the year were partial. A variable H then was obtained by dividing by the sample average. Differences in the slope of $\log(H)$ by county and treatment were tested in the model by including the two- and three-way interactions between $\log(H)$, CO and TR. The analysis was carried out using the SAS procedure GENMOD (SAS Institute Inc., 1999).

$\log(H)$ was checked for linearity by the inclusion of quadratic terms, and also by being supplemented by a categorical variable based on the percentiles and testing the extra term for significance. Tests were carried out to examine if the effect of YEAR was linear, before or during the study period. An assessment of the goodness-of-fit was obtained by examining residuals and by a Hosmer–Lemeshow test. The LOGISTIC procedure of SAS (SAS Institute Inc., 1999) which assumes an independence correlation structure on the repeated measures on a herd was used for the latter.

2.5.3.2. Survival analysis. A Cox regression model was constructed as described by Collett (1994). In this model, interest was centred on the hazard of restriction at any time from 1 September 1992. Herd size and YEAR were entered into the model as time-dependent variables, changing with chronological time. To account for herds with multiple restrictions, the time-dependent factor PH was included. The counting-process form of a Cox model was used with the Anderson–Gill method for treating multiple events (Therneau and Grambsch, 2000). Thus, the correct YEAR effects are used for second and subsequent survival times. Both the Wald and jackknife estimates of standard errors were examined (Therneau and Grambsch, 2000). The model included the terms TR, CO and PH and all two- and three-way interactions including those with YEAR. Terms were dropped from the model following hierarchical rules and using the likelihood-ratio test. Note that the effect of YEAR is subsumed in the baseline hazard function but not interactions with YEAR. The model was checked by examining the martingale, influence and Schoenfeld residuals. The effect of herd size was examined for linearity.

A similar Cox model also was developed to model survival in the 5-year period prior to the study and the 5-year period during the study. Model terms were included and tested as in the 10-year model, with the factor PERIOD replacing YEAR. This model was used to estimate the overall hazard ratio between removal and reference areas and between periods.

3. Results

3.1. The study areas and study herds

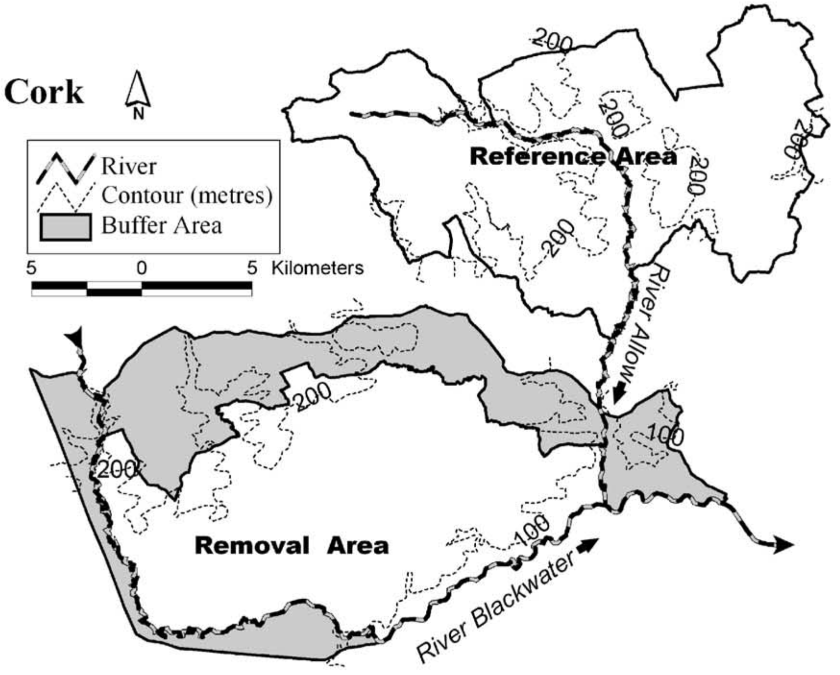
The total size of the study areas (excluding the buffer areas) was 1961 km^2 (Table 1), which is approximately 3.9% of the agricultural land area of the Republic of Ireland.

Table 1
Area (km^2) of the removal, buffer and reference areas in counties Cork, Donegal, Kilkenny and Monaghan (Republic of Ireland)

County	Removal	Buffer	Reference
Cork	188	119	199
Donegal	215	11	275
Kilkenny	252	61	253
Monaghan	305	63	274

3.1.1. The study areas

The study areas in Cork (with a total area of 387 km^2) lie in a major dairying area of Ireland, with a high proportion (89%) of pasture land and high grazing density (1.7 livestock unit (LU)/ha). The removal area was bounded to the south and east by the rivers Blackwater and Allow, to the north and west by a total of 119 km^2 of buffer areas (Fig. 2). A total of 87 badgers had been removed under licence from the Cork study areas prior to the start of the study (most during 1990–1993) with an average of 0.04 and 0.01 badgers/km^2/year during the 8 years prior to study start in the removal and reference areas, respectively. In the study areas of Donegal (covering a total of 490 km^2), the key landscape features are mountains, moors, heathland, bog and sea inlets, and with only 37% pasture land. In this region, stock-grazing density is low (1.0 LU/ha), cattle farming is predominantly suckler production, and herd sizes are small. The Donegal removal area was bounded by the sea and small (11 km^2) buffer areas to the south. A total of 133 badgers had been removed under licence from the Donegal study areas prior to the start of the study (the majority during 1990–1992) with an average of 0.07 and 0.003 badgers/km^2/year during the 8 years prior to study start in the removal and reference areas, respectively. The Kilkenny study areas (covering 505 km^2) are generally flat, and characterised in the main by rich pasture land divided by extensive hedgerow. In this area, cattle enterprises are mainly suckler and beef production, with the average herd size and grazing density (1.9 LU/ha) being larger than all other study areas. The Kilkenny removal area was bordered on the east by the river Barrow, the west and south by the river Nore, and by buffer areas (61 km^2 in total) to the north and southeast. A total of 301 badgers had been removed under licence from the Kilkenny study areas prior to the start of the study (the majority during mid 1995 to mid 1996) with an average of 0.10 and 0.04 badgers/km^2/year during the 8 years prior to study start in the removal and reference areas, respectively. The largest study area was in county Monaghan, covering 579 km^2. In this county, the landscape is dominated by rolling hills and pasture land, and intensive suckler production with high grazing densities of approximately 1.8 LU/ha. The Monaghan removal area was separated from Northern Ireland by the river Blackwater to the northeast and by a series of mountains including Slieve Beagh (380m) to the north west. There was a 63 km^2 buffer area to the south. Throughout the study period, there was no official removal of badgers in Northern Ireland. A total of 249 badgers had been removed under licence from the Monaghan study areas prior to the start of the study (the majority during mid 1994 to mid 1996 with an average of 0.09 and 0.01 badgers/km^2/year during the 8 years prior to study start in the removal and reference areas, respectively). The rivers Blackwater in counties Cork and Monaghan are separate Irish rivers.



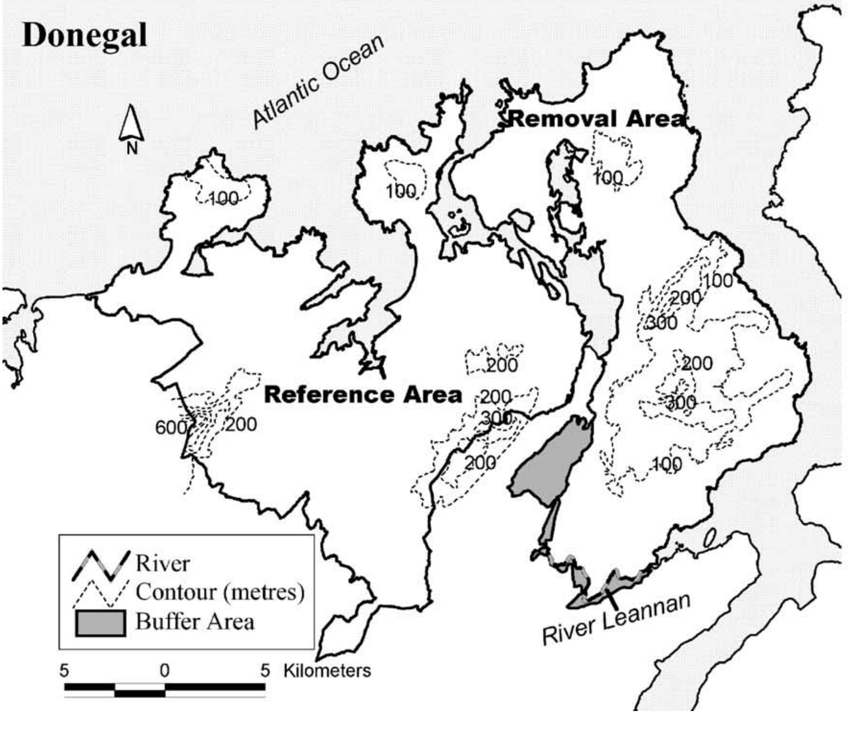


Fig. 2. The removal, buffer and reference areas within counties Cork, Donegal, Kilkenny and Monaghan, Ireland.

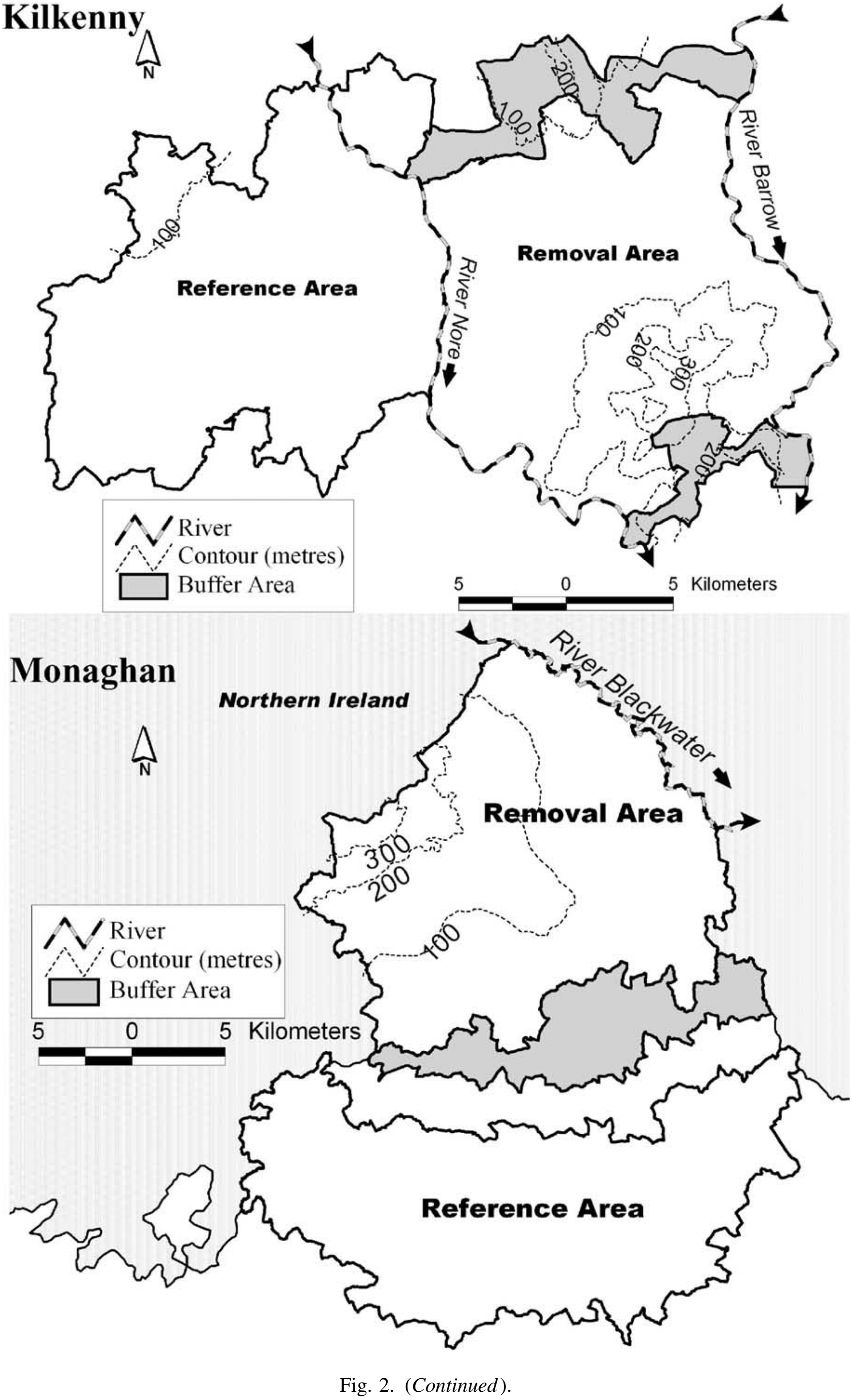


Fig. 2. (*Continued*).

3.1.2. Comparison of the matched reference and removal areas

The matching attributes for the removal and reference areas in each county are presented in Table 2. In the removal areas, the apparent prevalence of tuberculosis, measured as the mean number of tuberculin reactors per thousand animal tests (APT) during the 9 years prior to study start, ranged from 4.2 to 5.7. In contrast, the mean national APT during that period was 3.3. With the exception of Monaghan, the removal-area APT was higher than that in the corresponding reference area. In all counties prior to study start, the intensity of licensed badger removal had been higher in the removal compared with the reference areas. The highest average removal intensity during this period (in the Kilkenny removal area) was 0.09 badgers/km^2/year.

3.1.3. The study herds

There were 3280 herd owners with herds that met the definition of a study herd. This accounted for 76% of herd owners with at least some land within the study areas. Further,

Table 2
Attributes of the matched removal and reference areas in counties Cork, Donegal, Kilkenny and Monaghan

County, attribute	Removal area	Reference area
Cork		
APT (1987–1995)	4.9	4.0
Cattle population	32,252	27,533
Average herd size[a]	56	58
%Dairy herds	44.6	35.1
%Grassland	89.5	83.8
Grazing density (LUs[b]/100 ha)	167.5	159.6
Donegal		
APT (1987–1995)	5.7	4.3
Cattle population	16,819	10,660
Average herd size[a]	21	15
%Dairy herds	5.1	4.2
%Grassland	37.1	19.8
Grazing density (LUs[b]/100 ha)	106.0	120.3
Kilkenny		
APT (1987–1995)	4.2	3.7
Cattle population	31,508	41,951
Average herd size[a]	71	93
% Dairy herds	14.9	6.4
% Grassland	70.8	70.9
Grazing density (LUs[b]/100 ha)	189.1	193.2
Monaghan		
APT (1987–1995)	5.4	6.5
Cattle population	36,465	41,218
Average herd size[a]	31	37
%Dairy herds	17.9	24.8
%Grassland	73.2	94.9
Grazing density (LUs[b]/100 ha)	180.3	176.2

[a] Average number of cattle in each herd.
[b] Livestock units per 100 hectares.

the land owned by these farmers accounted for 71% of all land within the removal and reference areas that was owned by cattle farmers.

3.2. Badger removal

3.2.1. Badger-habitat survey

Permission to survey was refused only on 1 holding of 19 ha in Cork. During the initial survey, >5000 setts were identified, with a further 400 being found later in the study period. In total, 3077 and 2448 setts were identified in the removal and reference areas, respectively.

3.2.2. The programme of badger removal

Permission to undertake a programme of badger removal within the removal areas was refused at 13 surveyed setts (0.42% of those surveyed) and in the 19-ha area in Cork where no survey could be conducted. These 13 setts were located in Cork (5 setts), Donegal (5) and Kilkenny (3).

There were 5867 removal operations and 2360 badger removals in the removal and buffer areas during the study period (Table 3); 50.1 and 41.2% of all removals in these areas occurred during the first 12 months, respectively. In the reference areas, 258 badgers were removed following 64 severe outbreaks of bovine tuberculosis (as defined previously). This consisted of a total of 321 removal operations. The removal intensity in the removal, buffer and reference areas during the study is presented in Table 3, and reflects the relative badger density throughout the study period, assuming uniform removal efficacy. Removal intensity in the removal and reference areas during the first 2 years of the study averaged 0.57 and 0.07 badgers/km^2/year, respectively.

3.2.3. Infection status amongst badgers

Of the 2360 badgers captured in the removal and buffer areas during the study period, 2310 (97.9%) were examined *post mortem* with samples being forwarded for culture and/or histopathology. The remainder, in error, were examined *post mortem* only. Of the initial 2310 badgers, 450 (19.5%) were considered positive for tuberculosis. Of the 258 badgers captured in the reference areas during the study period, 218 (84.5%) were examined at *post mortem* with samples being forwarded for culture and/or histopathology. The remainder were examined at *post mortem* only. Of the former, 57 (26.1%) were deemed positive for tuberculosis. The prevalence of detected infection in the 2 populations was significantly different ($\chi^2 = 5.52$, d.f. = 1, $P = 0.02$).

3.3. Tuberculosis prevalence in cattle

3.3.1. Univariable analyses

3.3.1.1. Confirmed herd restrictions. In the removal and reference areas of counties Cork, Kilkenny and Monaghan during the study period, there was a general decline in the percentage of herds with confirmed restrictions. Apart from 2001/2002 in the reference area, there were few confirmed herd restrictions in the study areas of county Donegal. The observed prevalence of confirmed herd restrictions in the removal areas during the final year of the study ranged from 0.3% (Donegal) to 2.0% (Monaghan) compared with a range

Table 3
Number of badgers removed, and the removal intensity (badgers removed per km^2), in the removal, buffer and reference areas of counties Cork, Donegal, Kilkenny and Monaghan (Ireland) during 1 September 1997 to 31 August 2002

Year	Cork		Donegal		Kilkenny		Monaghan	
	No. of badgers	Removal intensity (removals/km^2)	No. of badgers	Removal intensity (removals/km^2)	No. of badgers	Removal intensity (removals/km^2)	No. of badgers	Removal intensity (removals/km^2)
Removal areas								
1997/1998	235	1.25	191	0.89	189	0.75	176	0.58
1998/1999	103	0.55	38	0.18	83	0.33	84	0.28
1999/2000	46	0.24	16	0.07	61	0.24	71	0.23
2000/2001[a]	29	0.15	16	0.07	28	0.11	34	0.11
2001/2002	36	0.19	16	0.07	49	0.19	78	0.26
Total	449	2.39	277	1.29	410	1.63	443	1.45
Buffer areas								
1997/1998	166	1.39	17	1.55	61	1.00	78	1.24
1998/1999	85	0.71	16	1.45	16	0.26	42	0.67
1999/2000	53	0.45	10	0.91	14	0.23	34	0.54
2000/2001[a]	22	0.18	6	0.55	18	0.30	20	0.32
2001/2002	31	0.26	16	1.45	33	0.54	43	0.68
Total	357	3.00	65	5.91	142	2.33	217	3.44
Reference areas								
1997/1998	18	0.09	0	0.00	7	0.03	4	0.01
1998/1999	36	0.18	9	0.03	43	0.17	21	0.08
1999/2000	23	0.12	0	0.00	3	0.01	17	0.06
2000/2001[a]	14	0.07	4	0.01	16	0.06	10	0.04
2001/2002	6	0.03	0	0.00	25	0.10	2	0.01
Total	97	0.49	13	0.05	94	0.37	54	0.20

[a] The number of badgers removed in the removal area in 2000/2001 was low due to restrictions imposed because of the foot-and-mouth disease outbreak in Ireland.

Table 4
Numbers of herds and percentage of these herds with confirmed restrictions for tuberculosis, in the reference and removal areas in four counties of Ireland during 1 September 1992 to 31 August 2002

Year	Treatment	Cork		Donegal		Kilkenny		Monaghan	
		Total herds	%Restricted	Total herds	%Restricted	Total herds	%Restricted	Total herds	%Restricted
1992/1993	Reference	265	3.4	369	2.2	215	7.0	533	9.8
	Removal	290	9.3	392	3.1	215	4.2	658	7.6
1993/1994	Reference	266	6.8	369	1.1	225	8.4	535	10.7
	Removal	292	7.5	396	4.0	233	3.9	650	7.5
1994/1995	Reference	270	9.6	374	1.1	231	6.5	538	8.9
	Removal	294	10.2	394	7.6	232	7.3	653	7.5
1995/1996	Reference	273	8.8	370	3.5	232	6.5	540	7.8
	Removal	293	12.3	390	3.6	231	12.6	668	6.3
1996/1997	Reference	270	13.3	362	0.3	232	8.2	545	5.5
	Removal	292	16.4	379	0.3	229	9.2	680	5.3
1997/1998	Reference	272	11.0	361	1.1	230	8.7	554	10.3
	Removal	288	10.1	375	0.8	230	6.1	687	2.8
1998/1999	Reference	271	16.6	349	1.4	222	12.6	565	11.0
	Removal	285	7.7	375	1.6	230	1.7	701	4.6
1999/2000	Reference	271	12.2	343	1.5	214	11.7	565	7.4
	Removal	282	3.9	375	0.8	229	2.6	681	3.5
2000/2001	Reference	274	4.4	334	1.2	213	5.6	559	6.8
	Removal	270	0.7	370	0.3	225	2.7	661	3.6
2001/2002	Reference	269	4.8	320	5.6	206	7.8	545	5.3
	Removal	259	1.2	365	0.3	214	1.9	644	2.0

of 0.3% (Donegal) to 16.4% (Cork) in the year prior to study start. In contrast, in the reference areas the observed prevalence of confirmed restriction during the final year of the study ranged from 4.8% (Cork) to 7.8% (Kilkenny) compared with a range of 0.3% (Donegal) to 13.3% (Cork) in the year prior to study start (Table 4).

3.3.1.2. Survival analysis. As an exploratory analysis, Fig. 3 displays the Kaplan–Meier survival curves in each county for the removal and reference areas during the study period. Note these do not take herd size into account. In every county, there was a significant shortening of survival time ($P < 0.001$ using the Wilcoxon test) for the reference areas. Table 5 gives the estimates of the 5-year survival probability from these curves. In each removal area, the probability of surviving for 5 years without a confirmed restriction was higher than the corresponding reference area ($P < 0.001$ in all counties, Wald's test).

3.3.2. Multivariable analyses

3.3.2.1. Logistic regression. There was a total of 3242 missing values (9.9% of the total possible values), a consequence of herds that did not have any stock in the year in question.

Of these, 3048 occurred before the first or after the last herd record, so that 194 out of a possible 29,752 (0.65%) values were missing between the first and last record for a herd.

There was a significant CO × TR × YEAR ($P < 0.001$) interaction indicating that the treatment effect varied by county and year. The interactions log(H) × CO × TR ($P = 0.13$), log(H) × CO ($P = 0.32$), log(H) × TR ($P = 0.80$) were insignificant, as were PH × CO × TR ($P = 0.85$), PH × CO ($P = 0.35$) and PH × TR ($P = 0.43$). No quadratic terms were required for log(H). Thus the effects of log(H) and PH were the same in all areas. These were significant ($P < 0.001$) with odds ratios (95% confidence bounds) 1.25 (1.14, 1.37) corresponding to a doubling of herd size, and 1.38 (1.21, 1.57) for previous history over no previous history, respectively. The estimated correlation parameter from fitting a model containing an AR1 correlation structure was 0.003. Independent correlation structure gave very similar results in terms of the parameter estimates and standard errors.

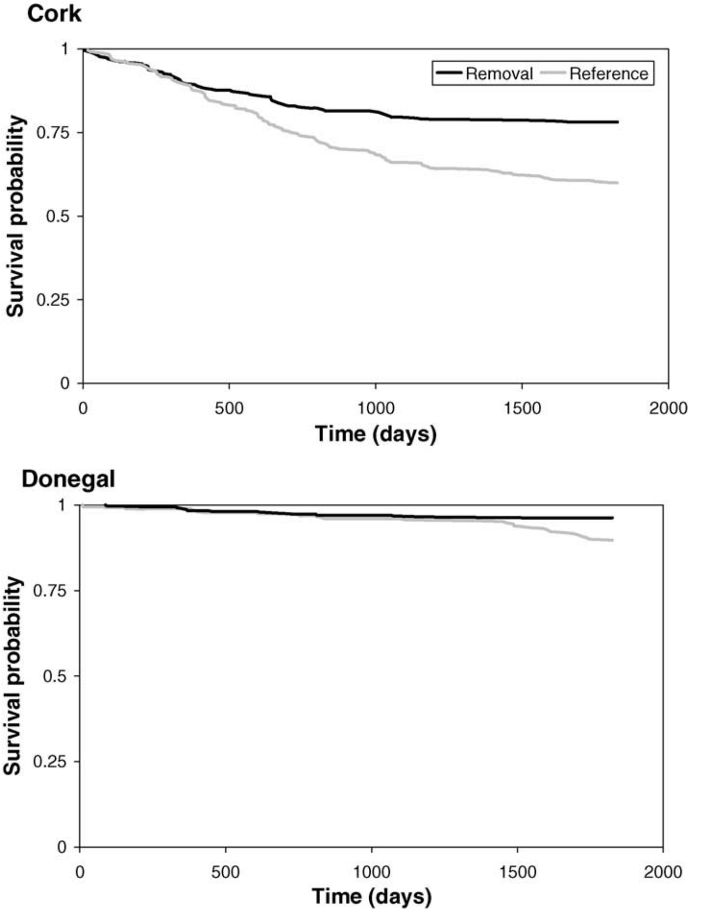


Fig. 3. Kaplan–Meier survival curves (until new herd restriction for bovine tuberculosis) for each county during the study period.

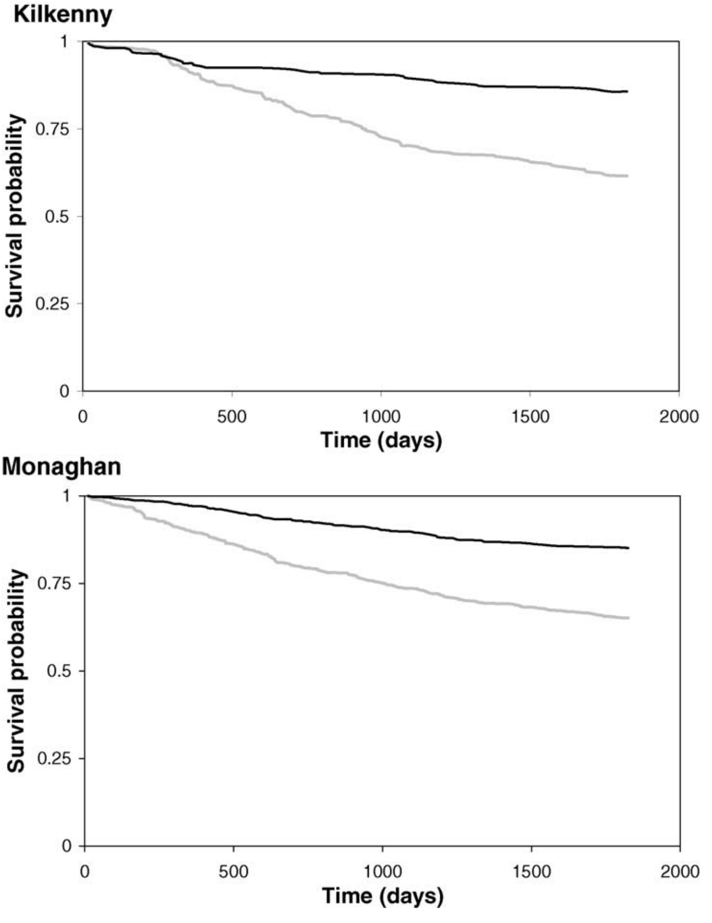


Fig. 3. (*Continued*).

Table 5
Kaplan–Meier probability of surviving for 5 years without a confirmed restriction, by county and treatment, in the study period

County	Reference area			Removal area		
	Survival probability	S.E.	No. in the risk set	Survival probability	S.E.	No. in the risk set
Cork	0.599	0.027	273	0.781	0.024	270
Donegal	0.897	0.016	331	0.963	0.010	370
Kilkenny	0.615	0.030	207	0.857	0.023	224
Monaghan	0.651	0.019	550	0.851	0.013	663
All counties	0.690	0.012	1360	0.865	0.008	1531

The Hosmer-and-Lemeshow goodness-of-fit test detected no lack of fit ($P = 0.73$). This test assumes independence correlation structure but was considered appropriate in view of the similarity between estimates from this and a model with AR1 correlation structure. Pearson residuals were examined using both an index plot and a half-normal plot with simulated envelope (Collett, 2002). There were no indications that the model was inappropriate.

The AR1 model will be considered as the final model because it does account for the small correlation present in the data. This included the terms: CO × YEAR, CO × TR × YEAR, log(*H*), PH.

The CO × TR × YEAR term (Table 6) compares removal and reference areas for all herd sizes and levels of PH. The parameterisation used means that a lower prevalence of restriction in the removal compared to the reference area corresponds to a negative term. During the 5 years of the study period, the odds ratios of prevalence of a confirmed restriction (from Table 6) were significantly <1 in at least 3 of the 5 years of the study period, the exception being Donegal where this was true of the last year. These results contrast with the pre-study period where odds in reference areas were not significantly different from the odds in the corresponding removal area, except in 2 years in Donegal and 1 year in Cork and Kilkenny, where the removal area had higher odds.

3.3.2.2. Survival analysis. The final model contained the terms PH, log(*H*), TR, CO and the two- and three-way interactions between TR, CO and YEAR. The TR × CO × YEAR interaction was significant ($P < 0.001$); therefore, the effect of treatment varied over counties and over years. The two- and three-way interactions between PH, CO and TR (PH × CO × TR, $P = 0.86$; PH × CO, $P = 0.24$; PH × TR, $P = 0.96$) and log(*H*) with CO and TR, (log(*H*) × CO × TR, $P = 0.16$; log(*H*) × CO, $P = 0.29$; log(*H*) × TR, $P = 0.22$), were not significant. In addition, the interaction terms PH × CO × YEAR ($P = 0.19$), PH × TR × YEAR ($P = 0.87$) and PH × YEAR ($P = 0.16$) were not significant. The Wald and jackknife estimates of standard error did not differ to two decimal places, so Wald estimates were used because they reduced computational time by a third. Table 7 compares the removal and reference areas for each county and each year. For example, an estimate of 1.25 is the difference (removal − reference) in the log–hazard function. Cork, Kilkenny and Monaghan all show significant effects of treatment during the study period, with hazard ratios <1. Donegal shows a significant effect of treatment only in the final year of the study, although the hazard ratio is decreasing in the last 3 years. There were significant differences between removal and reference areas prior to the study period (with removal having a higher hazard) for 1 year in Cork and Kilkenny and 2 years in Donegal. In 1 year in Kilkenny, the removal area had a significantly lower hazard. The effects of log(*H*) and PH were significant and positive ($P < 0.001$) (Table 7).

To summarise results, a Cox model was fitted with PERIOD replacing YEAR (Table 8). This reduced model contained the terms PH, log(*H*), TR, CO and the two- and three-way interactions between TR, CO and PERIOD. Thus, the effect of treatment varied over counties and over period. The hazard ratios (removal over reference) in the study period ranged from 0.28 to 0.52 and were significantly <1 in every county. In the pre-study period, the hazard ratios (removal over reference) were significantly >1 in Cork and Donegal while not significant in Kilkenny and Monaghan.

Table 6
Estimates (*b*) from the logistic GEE model, of the difference in the log odds and the odds ratio (OR) of a confirmed herd restriction for bovine tuberculosis in the removal area compared to the reference area for the four counties (Ireland)

Year	Cork				Donegal				Kilkenny				Monaghan			
	b	S.E.	*P*	OR	*b*	S.E.	*P*	OR	*b*	S.E.	*P*	OR	*b*	S.E.	*P*	OR
1992/1993	1.19	0.40	0.003	3.29	0.23	0.46	0.620	1.26	−0.38	0.44	0.389	0.69	−0.14	0.21	0.495	0.87
1993/1994	0.21	0.33	0.537	1.23	1.19	0.57	0.035	3.30	−0.66	0.42	0.117	0.52	−0.25	0.21	0.230	0.78
1994/1995	0.15	0.28	0.597	1.16	1.86	0.54	0.001	6.41	0.31	0.37	0.407	1.36	−0.04	0.21	0.870	0.97
1995/1996	0.44	0.28	0.114	1.56	−0.22	0.40	0.586	0.81	0.90	0.34	0.007	2.47	−0.08	0.23	0.738	0.93
1996/1997	0.34	0.24	0.157	1.41	−0.30	1.42	0.831	0.74	0.22	0.34	0.507	1.25	0.13	0.26	0.607	1.14
1997/1998	−0.03	0.28	0.914	0.97	−0.59	0.77	0.442	0.55	−0.28	0.37	0.445	0.75	−1.23	0.27	<0.001	0.29
1998/1999	−0.79	0.28	0.005	0.45	−0.14	0.61	0.820	0.87	−2.03	0.55	<0.001	0.13	−0.79	0.23	0.001	0.45
1999/2000	−1.16	0.36	0.001	0.32	−0.86	0.74	0.246	0.42	−1.44	0.47	0.002	0.24	−0.63	0.26	0.018	0.53
2000/2001	−1.77	0.77	0.022	0.17	−1.77	1.12	0.114	0.17	−0.61	0.51	0.232	0.54	−0.49	0.27	0.067	0.61
2001/2002	−1.40	0.65	0.031	0.25	−3.33	1.03	0.001	0.04	−1.35	0.57	0.017	0.26	−0.84	0.34	0.013	0.43
log(*H*)[a]	0.58	0.03	<0.001	1.79												
PH[b]	0.32	0.07	<0.001	1.38												

Annual estimates during 1 September 1992 to 31 August 2002; significant estimates ($P < 0.05$) shaded.

[a] Constant effect log herd size.

[b] Constant effect of previous history.

Table 7
Estimates (*b*) from the Cox model of the difference in the log–hazard function, and the hazard ratio (HR) of a confirmed herd restriction for bovine tuberculosis in the removal area compared to the reference area in four counties (Ireland)

Year	Cork				Donegal				Kilkenny				Monaghan			
	b	S.E.	*P*	HR	*b*	S.E.	*P*	HR	*b*	S.E.	*P*	HR	*b*	S.E.	*P*	HR
1992/1993	1.25	0.40	0.002	3.48	0.13	0.46	0.768	1.14	−0.42	0.44	0.338	0.65	−0.05	0.21	0.809	0.95
1993/1994	0.23	0.31	0.448	1.26	1.17	0.56	0.035	3.23	−0.85	0.42	0.043	0.43	−0.14	0.20	0.487	0.87
1994/1995	0.05	0.28	0.852	1.05	1.72	0.53	0.001	5.56	0.31	0.37	0.409	1.36	−0.17	0.20	0.393	0.84
1995/1996	0.29	0.27	0.286	1.34	−0.23	0.39	0.550	0.79	0.75	0.32	0.020	2.12	0.00	0.22	0.985	1.00
1996/1997	0.35	0.22	0.119	1.41	−0.31	1.41	0.828	0.74	0.21	0.32	0.517	1.23	0.11	0.25	0.669	1.11
1997/1998	0.03	0.26	0.906	1.03	−0.59	0.76	0.438	0.55	−0.28	0.35	0.414	0.75	−1.20	0.27	<0.001	0.30
1998/1999	−0.75	0.27	0.005	0.47	−0.14	0.61	0.820	0.87	−1.90	0.54	<0.001	0.15	−0.82	0.22	<0.001	0.44
1999/2000	−1.04	0.35	0.003	0.35	−0.85	0.73	0.247	0.43	−1.44	0.45	0.002	0.24	−0.72	0.26	0.006	0.49
2000/2001	−1.83	0.76	0.016	0.16	−1.72	1.12	0.124	0.18	−0.59	0.51	0.247	0.56	−0.52	0.27	0.051	0.59
2001/2002	−1.40	0.64	0.029	0.25	−3.24	1.03	0.002	0.04	−1.34	0.56	0.017	0.26	−0.93	0.34	0.007	0.40
log(*H*)[a]	0.52	0.03	<0.001	1.68												
PH[b]	0.25	0.06	<0.001	1.29												

Annual estimates during 1 September 1992 to 31 August 2002; significant estimates ($P < 0.05$) shaded.

[a] Constant effect log herd size.

[b] Constant effect of previous history.

Table 8
Estimates (b) from the reduced Cox model of the difference in the log–hazard function, and the hazard ratio (HR) of a confirmed herd restriction for bovine tuberculosis in the removal area compared to the reference area in four counties (Ireland)

Period	Cork				Donegal				Kilkenny				Monaghan			
	b	S.E.	*P*	HR	*b*	S.E.	*P*	HR	*b*	S.E.	*P*	HR	*b*	S.E.	*P*	HR
1992–1997	0.36	0.12	0.004	1.43	0.61	0.22	0.005	1.83	0.10	0.16	0.523	1.11	−0.07	0.10	0.480	0.93
1997–2002	−0.66	0.15	<0.001	0.52	−1.27	0.32	<0.001	0.28	−1.04	0.20	<0.001	0.35	−0.84	0.12	<0.001	0.43
log(*H*)[a]	0.51	0.03	<0.001	1.67												
PH[b]	0.25	0.06	<0.001	1.29												

The periods from 1 September 1992 to 31 August 1997 and from 1 September 1997 to 31 August 2002; significant estimates ($P < 0.05$) shaded.
[a] Constant effect log herd size.
[b] Constant effect of previous history.

Table 9
Estimates (b) from the reduced Cox model of the effect of period on the log–hazard function, and the hazard ratio (HR) of a confirmed herd restriction for bovine tuberculosis in the study (1 September 1997 to 31 August 2002) compared to the pre-study (1 September 1992 to 31 August 1997) periods in the reference and removal areas of four counties (Ireland)

Treatment	Cork				Donegal				Kilkenny				Monaghan			
	b	S.E.	P	HR	b	S.E.	P	HR	b	S.E.	P	HR	b	S.E.	P	HR
Reference	0.15	0.16	0.363	1.16	0.13	0.28	0.653	1.13	0.11	0.20	0.565	1.12	−0.15	0.16	0.363	0.86
Removal	−1.02	0.20	<0.001	0.36	−1.75	0.32	<0.001	0.17	−1.03	0.24	<0.001	0.36	−0.92	0.18	<0.001	0.40

Significant estimates ($P < 0.05$) have been shaded.

Table 9 examines the difference between the pre-study and study period. In the reference areas, there was no significant change between the two periods for any county. In contrast, in the removal areas the hazard ratio in the study period compared to prior to the study was <1 in every county, indicating that survival times for the removal area in every county were longer in the study period than before.

There was also a significant county effect ($P < 0.001$) in the reference areas, in the pre-study period and again in the study period, and in the removal areas in the pre-study period and again in the study period.

A plot of the martingale residuals versus the linear predictor was obtained for the Cox yearly model. The plot showed the usual distinction between censored and uncensored observations. There were no outliers. (We note that deviance residuals are available only for models with fixed covariates.) Plots of the dfbeta residuals (influence residuals) for both PH and log(H) were examined. The influences were small, $\leq$1/15 of a standard error for PH and 1/9 of a standard error for log(H). Plots of the weighted Schoenfeld residuals versus the linear predictor, versus time and versus herd index were also examined and showed no evidence of model misspecification.

4. Discussion

4.1. Study justification

The Irish bovine tuberculosis-eradication programme began in 1954. Although there was initial programme success (with animal prevalence falling from an initial 17–0.3% in 1965), the programme has stalled with national animal prevalence remaining relatively stable. During this period, the disease has remained geographically clustered, with herd prevalence at almost twice the national average in some counties. These findings are at odds with the British experience, where there has been a very-recent substantial increase in herd incidence and geographic spread (DEFRA, 2004). During the period 1988–1992, and in addition to a comprehensive national disease-eradication programme, the Irish government implemented (without any marked success) a number of strategies to improve progress towards bovine-tuberculosis eradication. These included exhaustive tuberculin testing (about 44 million tests were carried out in the years 1988–1991 on the national herd of approximately 7 million cattle), the setting up of a dedicated service to transport reactor animals to the slaughter plant as soon as they were identified, improved compensation payments for farmers, the setting up of a unit to undertake extensive research into the epidemiology of tuberculosis, greater controls on animal movement, improved cleansing and disinfection procedures on infected farms, extension of the restriction periods for infected herds and depopulation of heavily infected herds (Downey, 1992). The current trial was a logical extension of this earlier work and sought to clarify the role of infected badgers as a source of tuberculosis on Irish cattle farms.

4.2. Study design

Based on experiences from the East Offaly project (Eves, 1999), there is the potential for considerable badger immigration following the removal of badgers from a defined area. For this reason, we sought to bound the removal areas with natural geographic features (such as large rivers, mountain ranges and sea inlets) to limit inward migration of badgers during the study period. Because there are few sites in Ireland meeting this and each of the other selection criteria (higher-than-average disease prevalence, representative of the diverse Irish landscape), we elected to use purposive (that is, non-random) sampling to identify suitable removal areas and matched reference areas. In three of the four removal areas (all but Monaghan), immigration of badgers was minimised through effective geographic boundaries. Some limited immigration did continue during the study period, given the location of badger removals in the removal areas during the latter years of the study, as well as the higher intensity of removal in the buffer as compared with the removal areas (Table 3). However, this was unavoidable due to the presence of road bridges across key rivers and movement across buffer areas. In the north east of the Monaghan removal area, the boundary was less secure, being separated from Northern Ireland (where there was no official badger removal programme) by the narrow River Blackwater. As illustrated in Table 3, removal intensity during the last year of the study was higher in the Monaghan removal area than in the other counties. Regardless, the impact of limited immigration was minimal, due to the ongoing removal efforts throughout the study period. Any ongoing immigration of badgers (and associated cattle breakdowns) in the removal areas will have had the effect, if any, of increasing the prevalence of bovine tuberculosis in each removal area, and thereby underestimating the true effect of badger removal in this study.

Further, during each of the 5 years prior to study start in Monaghan, and at least 3 of the 5 years prior to study start in Cork, Donegal and Kilkenny, there was no significant difference in the probability of, or time to, a confirmed restriction in the matched removal and reference areas (Tables 6 and 7). This was despite some reduction of the badger populations, particularly in the removal areas of counties Monaghan and Kilkenny, during the 12 months from mid 1995 to mid 1996 (2 years prior to study start). During most years when there was an observed difference in the rate of herd restriction, the time to a confirmed restriction in each situation was significantly greater in the removal as compared with the matched reference area (Table 8). Therefore, if there were any residual differences between these matched study areas, they would have reduced the probability of detecting any treatment effect, if present. In each county, there was no significant difference in the probability of or time to a confirmed restriction between the matched areas during the full year (1 September 1996 to 31 August 1997) prior to study start (Tables 6 and 7).

The current study is based on an assessment of two different levels of badger removal on the prevalence of tuberculosis in cattle in Ireland. In the removal areas, badger removal is proactive (that is, the badgers were removed independent of and prior to any outbreak of bovine tuberculosis). Further, we sought to achieve as complete a removal as possible, and to sustain this throughout the study period. Although restraints do not effectively capture young badgers, this would not have adversely affected removal effectiveness, due to the ongoing efforts to remove adult badgers from each removal area. In contrast, badger removal in the reference areas was conducted reactively, in response to severe outbreaks of tuberculosis in cattle herds where the source reasonably could be attributed to badgers. To minimise capture intensity in these areas, the criteria for badger removal were more stringent than routinely used throughout Ireland; in the reference areas, badgers were removed only if there were four or more standard reactors and badgers could be implicated—whereas throughout the rest of the Republic of Ireland (apart from the removal and buffer areas in this study) badgers were removed if there were two or more standard reactors and badgers could be implicated. Therefore, the badger population in the reference areas was less disturbed than throughout most of the rest of the Republic of Ireland. To illustrate further, during the first 2 years of the study period, the intensity of badger removal in the reference area was equivalent to 0.07 badgers removed/km^2/year. In comparison and during the same period, the intensity of badger culling in the removal areas was 0.57 badgers/km^2/year (Table 3).

There are several reasons why we elected to use a reactive, rather than a no-removal, control. Reactive removal represented official government policy at the time. Therefore, use of a reactive, rather than no-removal, control is in keeping with standard scientific practice, where the comparison group generally should represent existing standard practice (Dohoo et al., 2003). Further, because it was contrary to official government policy and popular views about this disease, the use of a non-removal control might have encouraged the illegal removal of some badgers within the reference area. This latter problem, and the opposing effect of interference to removal, were extremely rare.

To minimise measurement bias, the matched removal and reference areas in each county were supervised by the same field people within the same administrative unit. In addition, the project was coordinated nationally, and all work was conducted in accordance with nationally agreed protocols (for field and laboratory work). As part of national coordination, regular meetings were held among field staff from each of the project areas.

4.3. Study findings

During the study period, there was a significant difference between the removal and reference areas in both the probability of and the time to a confirmed herd restriction due to tuberculosis. To illustrate, in the final year of the study the odds of a herd restriction in the removal as compared to the reference areas was 0.25 (95% C.I. 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 (Table 6). Further, the estimated probability of surviving 5 years without a confirmed restriction was between 7% (Donegal) and 24% (Kilkenny) higher in the removal over the reference area (Table 5). In the final year of the study, the hazard ratios (removal over reference) ranged from 0.40 to 0.04 or less (Table 7), a 60 to 96% decrease in the rate at which herds were becoming the subject of a confirmed restriction. These effects are consistent across all four counties, which is remarkable given known differences between each of these four areas. These differences include the varying levels of badger disturbance prior to study start (minimal in counties Cork and Donegal but recent in counties Kilkenny and Monaghan), varying levels of herd restriction in the pre-study period and the diverse farming environments and likely badger densities.

Donegal differs from the other counties. In both the removal and reference areas, the percentage surviving 5 years in the study period is 90% and over. In this county, there were very few confirmed restrictions in either area in the 6 years 1996/1997 through 2001/2002, with the exception of 18 restrictions in the reference area in 2001/2002. Low levels had been achieved in the two areas in the county prior to removal, and these persisted apart from this exception.

4.4. Statistical issues

The significant effect of county in modelling has reflected the clustered nature of the data. In this situation, it is common to consider a random effect (McDermott et al., 1997). However, in this study the purposive selection of the four removal areas and the matching reference areas would not justify this approach. It would be unsafe to assume the county effect for the selected areas behaved like randomly selected areas from geographical areas in Ireland. In such situations, it is recommended that a fixed-effect factor should be used (McCullogh and Searle, 2000, Section 1.6). Schukken et al. (2003) cites a similar example involving BHV1 data where three areas were selected purposively and states that the use of random effects is not appropriate. Moreover, because there were only four areas, the estimation of variation at this level is questionable and can be unstable (Schukken et al., 2003). In addition, the purpose of the present study was to determine if there were circumstances in Ireland under which levels of herd restriction changed following badger removal. Results from individual areas are therefore important in suggesting where removal might be beneficial, and of conditions in Ireland or elsewhere where the control of *M. bovis* in badgers should be considered.

Logistic regression and survival analyses both were conducted to obtain estimates of odds and hazard ratios. These estimates were similar, showing consistency of results from these two analyses (Tables 6 and 7). This is to be expected (Allison, 1995, page 233; Therneau and Grambsch, 2000, page 185).

Although time to infection is preferred over time to restriction, the former data are not known in a field-based control programme. We elected to limit our analyses to confirmed (as opposed to all) restrictions to minimise the probability of false positives. For the logistic analysis, the prevalence (rather than incidence, as in the survival analysis) of confirmed restriction was used to avoid missing values. Incidence values were missing—and would not have occurred at random—for all herds that were restricted at the start of any year, and remain restricted for a further 12 months. Also, giving a confirmed restriction status to the herd acknowledged the ongoing presence of risk factors contributing to the continued restriction.

Many factors increase the risk of a herd breakdown, including the bovine tuberculosis history of the area of the country, herd size, and the past tuberculosis history of the herd (Olea-Popelka, 2002). We controlled for the first factor by using APT as a matching criterion during the selection of the removal and reference areas in each county, and the latter factors were controlled during analysis. In keeping with previous findings (Griffin et al., 1996; Olea-Popelka, 2002; O'Mairtin et al., 1998a, 1998b; O'Sullivan and O'Keeffe, 1998), increasing risks and hazards in our study were associated with increasing herd size and with a history of a previous confirmed breakdown. Note that the relationship between breakdown risk and herd size is not entirely biological. When evaluating the status of herds based on imperfect tests applied to individual animals, there is an increased likelihood of at least one test-positive result in a disease-free herd as herd size increases (Martin et al., 1992).

4.5. Study validity

In a recent paper, Donnelly et al. (2003) anticipated the findings of the current study. These authors report an apparent increase in incidence of tuberculosis in cattle following reactive culling in the UK, and speculate that this effect is associated with disruption of the social organisation of badgers—leading, ultimately, to increased transmission of tuberculosis from badgers to cattle. Relevant to the current study, Donnelly et al. suggest that a similar effect results in an over-estimate of the true effectiveness of widespread proactive culling. However, in our study, there is no evidence in support of such an effect. Firstly, there is no statistically significant increase in levels of tuberculosis in cattle in response to reactive badger removal in the reference areas. As illustrated in Table 9, the difference in the time to a confirmed herd restriction in each of the reference areas in the pre-study compared to the study periods is not statistically significant. Secondly, throughout the study period, the intensity of badger removal in the reference areas was very low. During the first 2 years of the study (when most badger removals occurred), removal intensity was 0.07 badgers/km^2/year, which equates with an annual average of 17.3 badgers per county or 1 removal per 14.5 km^2. Any relationship between badger removal (at this level of removal intensity) and bovine tuberculosis would seem implausible. Nonetheless, we currently are investigating the possibility of spatial relationships between badger removal and confirmed herd restriction in the reference areas. For comparison, and based on available data (Donnelly et al., 2003), a total of 2047 badgers were removed from the 9 'reactive' triplet areas (A–I; each approximately 100 km^2) during a variable (average period of enrolment of 2.6 years) period from 1999 to 2003, which equates to an average removal rate of 0.87 badgers/km^2/year. Finally, any causal link between badger removal and bovine tuberculosis is certain to be supported by a time-lag sufficient to enable a defined chain of events to occur. These events would include disruption in badger social organisation leading (in a manner currently undefined) to increased transmission of tuberculosis among badgers, contact between cattle and the disturbed badger population leading to cattle exposure, the establishment of infection in cattle, the development of responsiveness to tuberculin following establishment of infection, the holding of the annual herd test (in the Republic of Ireland, each 12 months but at a time nominated by each farmer), the inspection of reactor animals at slaughter and laboratory confirmation of disease. Similar comments have been made elsewhere (Godfray et al., 2004). In our study, there was no evidence of a lagged temporal association between badger removal and confirmed restrictions in cattle in the reference area.

The study sites were not chosen randomly, for reasons given previously. Rather, we used a number of criteria during the selection of the study areas, including areas with recurrent problems of tuberculosis in cattle, sites that were suitable for the establishment of 'secure' removal areas, and areas that were reflective of the diverse farming environments found in Ireland. Consequently, the results from this study must be generalised with care. In Ireland, bovine tuberculosis is a clustered problem; to illustrate, 69 and 64% of the herd breakdowns during 1998–2000 occurred in 32 and 28% of the agricultural land in the north and south of the Republic of Ireland, respectively (O'Keeffe et al., 2002). It would seem reasonable – given the absolute size of the study areas (covering 1961 km^2 and representing 3.9% of the agricultural land area in the Republic of Ireland), the diversity of farming environments represented in this study and the general consistency of the key study results in each of the four areas—that these results can be generalised to other 'problem' areas of Ireland. This would concur with anecdotal veterinary field evidence. These results also may be generalised, with care, to other regions after considering differences in farming systems and badger ecology (such as population densities and preferential sett location).

Although feasible, we acknowledge that widespread badger removal is not a viable strategy for the long-term control of tuberculosis in the Irish cattle population. Therefore, an alternative strategy for reducing badger-to-cattle transmission of *M. bovis* in Ireland—namely, the development of an effective vaccine for badgers, is currently being investigated.

5. Conclusions

In conclusion, this study was conducted in four counties of Ireland where the incidence of tuberculosis in cattle herds at study start was higher than the national average. The counties are representative of many of the farming environments in Ireland. During the 5-year period of badger removal, the odds and hazard ratios of a confirmed restriction in the removal areas were significantly lower than in matched reference areas. We believe it is reasonable to attribute this effect specifically to proactive badger removal.

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