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A case study of bovine tuberculosis in an area of County Donegal, Ireland

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A descriptive analysis, to investigate the potential risk factors that might have contributed to the increased incidence of bovine tuberculosis (BTB) herd-breakdowns in the reference area of Co. Donegal during the fifth year of the four-area project (FAP), was performed. 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 nine 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, 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. The analysis supports the hypothesis that BTB in herds is a problem that cannot be addressed successfully by dedicating our efforts to the elimination of single risk factors. Neither is it a problem that needs to be investigated only at the herd level, but rather at the area level, including groups of contiguous herds.

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Introduction

A programme for the eradication of bovine tuberculosis (BTB) in cattle was initiated by the Irish government in 1954. The scheme, based on a test-and-slaughter policy, made considerable early progress, and Ireland attained BTB-free attestation status in 1965. However, further progress towards eradication has proved difficult – an experience shared with other developed countries (Ó Máirtín *et al.*, 1998). Current constraints to the eradication of BTB appear to be related to animal production practices and to the existence of an important wildlife reservoir for *Mycobacterium bovis*; namely the badger (*Meles meles*).

Several studies have been conducted to assess the impact of badger removal on the levels of BTB in cattle herds, including the 'east Offaly

project' (Ó Máirtín *et al.*, 1998) and 'four-area project' (FAP; Griffin *et al.*, 2005). The latter project, conducted in counties Cork, Donegal, Kilkenny and Monaghan between September 1, 1997 and August 31, 2002, was based on comparing the observed BTB incidence in herds located in matched 'removal' areas (where badger removal was proactively conducted throughout the period) and 'reference' areas (where a minimal level of focal badger removal was undertaken, in response to defined BTB herd breakdowns). During the first four years of the study, BTB herd breakdowns in the reference area in County Donegal ranged from nine to 18 per year. However, this situation changed markedly during the fifth year, during which 32 breakdowns were observed. In this paper, we present a descriptive analysis of these breakdowns with the aim of identifying risk factors that might have contributed to this increased incidence of BTB breakdowns.

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Material and methods

The 'reference' area in Co. Donegal covers 275 km². The main landscape features include mountains, moors, heathland, bogs and sea inlets, with only 37% of the area used as pasture land. The grazing density of stock is low (1.0 livestock unit/hectare), and cattle farming is predominantly beef-suckler production with small herd sizes (average herd size=15) (Centre for Veterinary Epidemiology and Risk Analysis, 2004). This paper focuses mainly on events from September

Table 1: Number of BTB herd breakdowns in each year, breakdowns characteristics and infected herds characteristics, Co. Donegal, September 1997 to August 2002, Ireland

	During each year of the study				
	1st year Sept 97-Aug 98	2nd year Sept 98-Aug 99	3rd year Sept 99-Aug 00	4th year Sept 00-Aug 01	5th year Sept 01-Aug 02
Overall					
Total number of BTB breakdowns	14	9	18	9	32
Number of breakdowns with at least one standard reactor	12	8	16	9	28
Number of breakdowns with at least one animal with detected lesion(s)	4	5	5	4	19
Number of breakdowns that were eligible for an ER76 investigation (with at least two standard reactors and a lesion)	5	6	6	5	21
Per breakdown					
Median herd size	24	22	26.5	25	22
Median number of reactors	1	1	1	1	1
Median number of standard reactors	1	1	1	1	1
Median number of animals with detected BTB lesion(s)	0	1	0	0	0.5
Days since the last full herd test	403	359	364	379	336

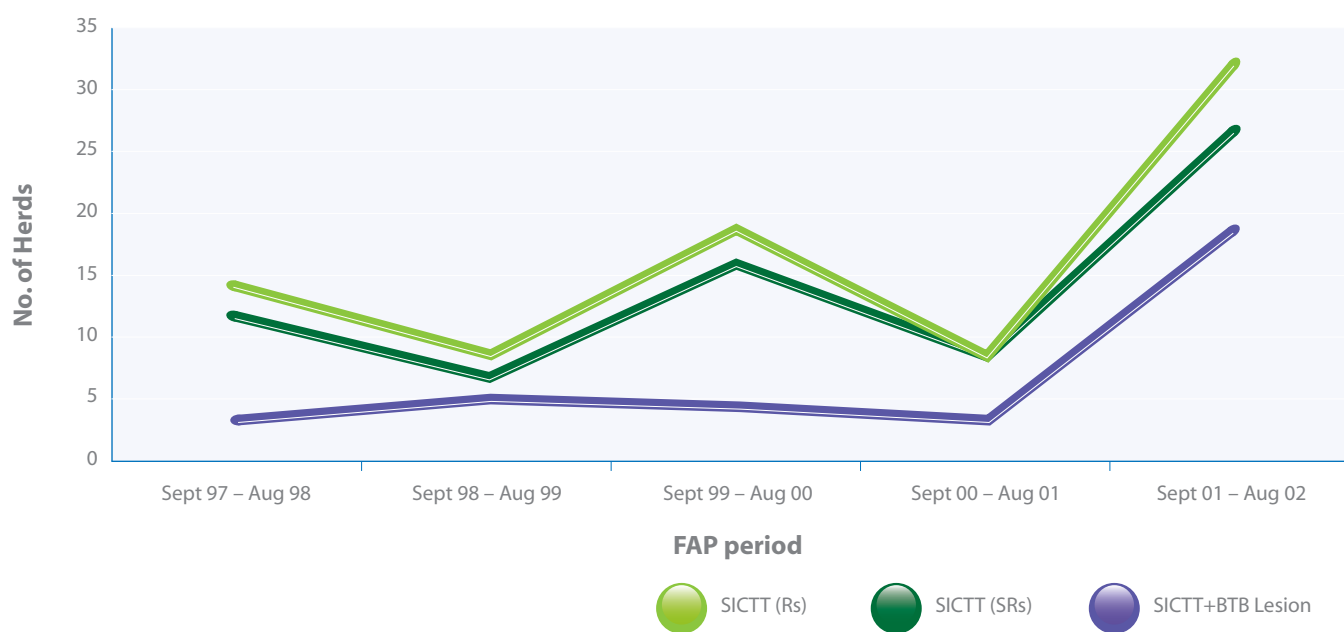


Figure 1: Distribution of BTB breakdowns, BTB breakdowns characteristics and year during the four-area project in the reference area in Co. Donegal.

1, 2001 to August 31, 2002. However, data from earlier years were used, as appropriate, to provide a context for the current study.

Four different data sets were used. Firstly, a dataset was obtained from the District Veterinary Office (Raphoe, Co. Donegal) with information on all (n=82) BTB herd breakdowns in the Donegal reference area during the five years of the four-area project, including:

- * Herd size and class of animals in the herd; and,
- * Herd BTB testing history including, for each test, the date, the reason for the test, the testing veterinarian, the number of animals tested, the number of BTB-reactor animals (for each reactor: age, sex, whether the animal was classed as a 'standard reactor' to the single intradermal comparative tuberculin test (SICTT), whether a BTB lesion was detected at slaughter and, if so, the site(s) of the lesion).

Secondly, the Cattle Movement Monitoring System (CMMS) database was used to obtain information related to animal introductions onto each farm.

Thirdly, the epidemiological investigation reports (known as the ER76 form) were examined on all BTB breakdowns involving at least two 'standard' reactors and at least one reactor with a detectable lesion to obtain details related to farm management practices.

Fourthly, a Geographical Information System (GIS) dataset was obtained from the Centre for Veterinary Epidemiology and Risk Analysis (CVERA) to identify the location of each herd (and its neighbours).

Table 2: Characteristics of the herds and their breakdowns during the fifth year of the four-area project in Co. Donegal, Ireland

Herd ID	Reason for test	BD date	Days between SICTT	Animals tested	Reactor animals	BTB lesions	BTB history	Purchased BTB infection	No. neighbors infected (%)
Group 1									
1	RND	14SEP/01	294	15	1	0	-	-	3 (0)
2	RND	14SEP/01	351	27	1	0	1997 II Inc	-	2 (0)
3	RND	20SEP/01	357	81	2	2	-	-	6 (0)
4	RND	27SEP/01	406	33	5	2	-	-	3 (0)
5	INC	02NOV/01	427	26	1	0	1998 2Sr	-	5 (0)
6	FLT	23NOV/01	315	23	1	0	-	-	4 (0)
7	INC	21DEC/01	406	13	1	0	-	-	3 (0)
8	RND	12JAN/02	390	7	1	0	-	-	3 (0)
9	FLT	25JAN/02	77	30	0	0	-	-	4 (0)
10	SCT	07FEB/02	427	41	1	0	2000 II Inc	-	7 (0)
11	FLT	08MAR/02	175	25	0	0	-	-	1 (0)
12	RND	23AUG/02	343	10	1	0	-	-	1 (0)
13	RND	30AUG/02	294	49	1	0	-	-	10 (0)
			Median	351	1	0			
Group 2									
14	RND	07SEP/01	372	12	1	0	-	-	4 (100)
15	RND	28SEP/01	490	48	1	1	-	Yes	4 (50)
16	RND	07JAN/02	360	15	3	1	-	-	1 (100)
17	FLT	31JAN/02	76	12	0	0	-	Yes	-
18	RND	26APR/02	287	46	1	0	-	-	9 (22)
19	CT	19APR/02	224	30	3	0	-	-	6 (67)
20	CT	10MAY/02	267	6	3	2	-	-	5 (60)
21	CT	10MAY/02	246	16	3	3	-	-	7 (71)
22	CT	30MAY/02	273	12	1	1	-	-	2 (50)
23	RND	06JUN/02	307	11	1	1	-	-	3 (67)
24	CT	14JUN/02	336	19	3	2	-	-	5 (20)
25	RND	24AUG/02	352	15	1	1	-	-	2 (0)
			Median	297	1	0			
Group 3									
B	RND	26OCT/01	533	22	1	1	1999 ISr	-	3 (33)
C	RND	17JAN/02	370	14	4	2	-	-	4 (50)
D	CT	14FEB/02	160	19	2	0	2001 II Inc	-	6 (100)
E	RND	16APR/02	319	26	3	1	-	-	3 (100)
X	CT	26APR/02	420	93	17	9	-	-	7 (71)
F	FLT	17MAY/02	77	34	1	1	-	-	5 (80)
			Median	344.5	2.4	1			

In an earlier analysis of the four-area project (Griffin *et al.*, 2005) only confirmed breakdowns (that is, those breakdowns where at least one lesion was detected at slaughter) were considered. Here, both confirmed and unconfirmed BTB breakdowns are described. For each breakdown the total number of reactors (R), and within the reactor animals the number of 'standard reactors' (Sr) and the number of reactor-animals with BTB lesions (L)* were counted. In Ireland, an animal is classified a standard reactor to the SICTT when the bovine reaction is >4mm larger than the avian reaction, or if local clinical signs (such as oedema, exudation, necrosis or pain) are present at the site of injection of the bovine tuberculin (Monaghan *et al.*, 1994). An animal is considered to be an 'inconclusive reactor' if the bovine reaction is between 1mm and 4mm greater than the avian reaction; inconclusive reactors may subsequently be reclassified 'reactors' if the field veterinary inspector considers this appropriate. Based on formal Department of Agriculture and Food instructions, field veterinarians should re-classify inconclusive responders as reactors if two or more standard reactors are identified during a herd test. In addition, field veterinarians may apply a progressively more severe interpretation to a test if such a course of action is agreed with the local state veterinarian with responsibility for the area. Where the majority of a group of animals fail the test, in-contact cohorts may be deemed reactors, even though they may be test-negative to the SICTT (Good *et al.*, 2003).

* BTB-like lesions are identified in only a proportion (about 30%) of reactors at routine meat inspection (lesions are detected more frequently in standard reactors as compared to reactors) (Byrne, 2000). In addition, a herd can be classified as BTB-positive if, under routine slaughter surveillance, an animal is found to have a BTB lesion confirmed by culture or histopathology (Costello *et al.*, 1998a).

Results

Overview

During the five years following September 1, 1997, 72 different herds were restricted for BTB, including 10 that were restricted twice, resulting in a total of 82 BTB herd breakdowns. **Figure 1** shows the temporal distribution of all BTB breakdowns. During the first four years of the study, there were four or five confirmed BTB herd breakdowns per year, and all were detected with SICTT testing. In the fifth year of the study, there was a substantial increase in the number of BTB breakdowns (n=32) including 19 confirmed breakdowns. Five of these breakdowns were initially detected as a result of surveillance during meat inspection. A number of testing veterinarians were involved in the testing of the BTB-positive herds.



Figure 2: Spatial-temporal distribution of the BTB herd breakdowns in reference area Co. Donegal, 1997-2002.

Table 1 summarises some characteristics of the 82 BTB breakdowns. The spatial-temporal distribution of the BTB breakdowns over the study period is presented in **Figure 2**. During the first four years, breakdowns were mainly isolated events distributed across the study area. However, of the 31 BTB-positive herds identified during the fifth year, 18 were immediate neighbours, forming a cluster of infected herds within an approximate radius of 1.8km (**Figure 2**; circled).

The following discussion concerns the spatial cluster of 18 herds in the eastern section of the reference area; 12 denoted as group 2 herds (**Table 2**). With a herd size of 93, herd 'X' (**Figure 3**) held the largest number of animals in this cluster and the largest number ($n=17$) of BTB-positive animals (reactors), including 16 standard reactors and nine animals with detected lesions. This herd had seven immediate neighbors, including five (71%) which were BTB-positive during year five; this cluster of six infected herds is referred to as group 3 herds (**Table 2**). Herd X was classified as BTB-positive on April 26, 2002 as part of a contiguous herd test (see below for details) because a neighbour's herd (herd 'E' in **Figure 3**) had a BTB breakdown, in a round test (in Ireland, this is the annual screening test for TB), 10 days prior to this date. The time-sequence and reason for testing herds around herd X that were identified as BTB-positive are shown in **Figure 4**. Before herd X was declared infected, four other neighbouring herds (referred to as herd 'A', 'B', 'C' and 'D' in **Figures 3** and **4**) were classified as BTB-positive. Herd A was the last BTB-positive herd identified during the fourth year of the FAP (August 30, 2001), one day before the fifth year began and 8.5 months before herd X was restricted. Herd A was identified as positive in a round test that disclosed one reactor and one standard reactor; both animals had BTB lesions at slaughter. Herds B, C and D were restricted in October 2001, January 2002 and February 2002, respectively (**Figure 4** and **Table 2**).

Although herd X was an immediate neighbour for herds A, B, C and D, a contiguous SICTT test was not performed at the time of testing these other four herds. Herd E, another neighbour, was restricted on April 16, 2002 on a routine round test with a cumulative total of seven reactor animals; four of them standard reactors, two with BTB lesions. Herd E was tested three months after herd C (319 days after its previous test) and the testing of herd X occurred 420 days after its previous herd test (March 2, 2001) (**Figure 4** and **Table 2**). The ER76 report indicated that all the grazing fragments of herd X were grazed by infected cattle. Also, it was noted that contact across farm boundaries or fences with neighbour's cattle was possible. Shared equipment with other farmers was practised in this herd also.

Table 2 shows the details for each of the 31 BTB-positive herds during the fifth year. Three groups of herds were created according to their spatial location. Group 1 included spatially isolated herds, group 2 included a subset of 12 herds within the 18-herd cluster and group 3 included herd X and its five infected neighbours during the fifth year. The median number of cattle tested per herd was 26 for group 1, 15 for group 2 and 24 for group 3.

Most likely source of infection

The following provides a summary of findings concerning the likelihood of infection from various sources, including factors such as the geographical location of each infected herd, their previous BTB history, their purchase of cattle, the BTB status of neighbouring herds and badger data for this area (**Table 2**). Herds in group 3 are identified by letters, all others by numbers. (Note: **Table 2** describes the 31 herds that broke down during the fifth year, thus herd A is not listed).

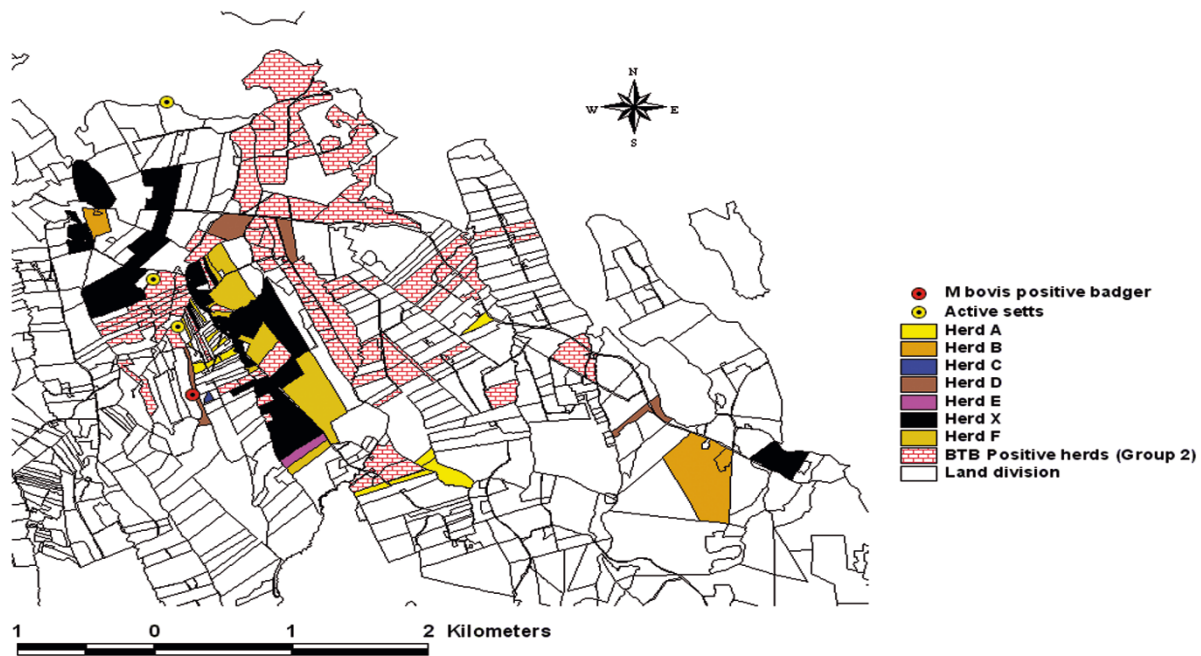


Figure 3: Location of BTB 18 infected herds belonging to the cluster during the fifth year of the four-area project.

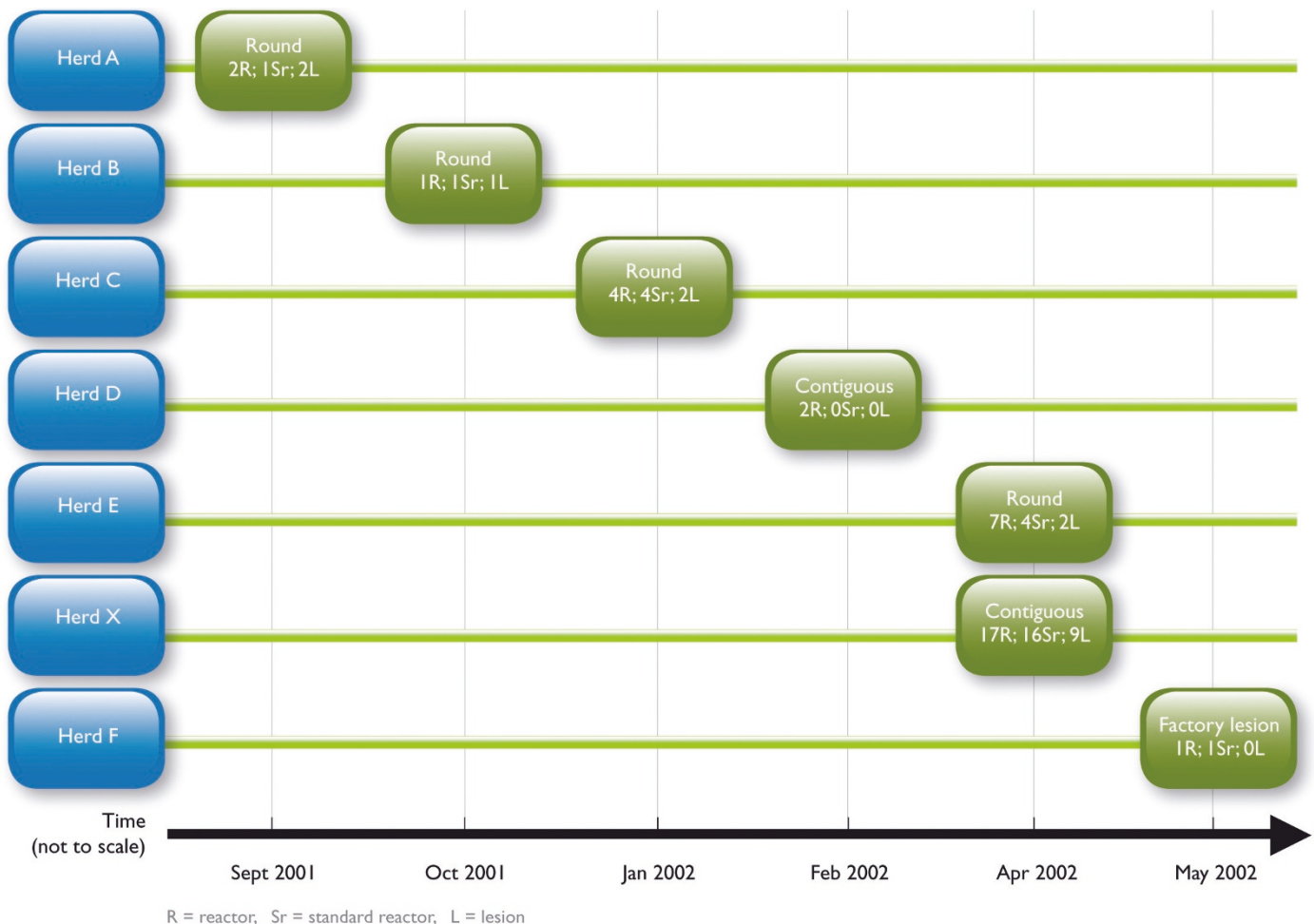


Figure 4: Herd testing time sequence and results for herd X and its neighbours during the fifth year of the four-area project.

Residual infection

Most (n=26) of the herds did not have a history of previous BTB events during the five-year period of the FAP. Among the remaining five herds, two had experienced a previous breakdown where standard reactors were detected, whereas three had breakdowns where only inconclusive reactors were detected. Herd X had not experienced a BTB breakdown since 1986. Two other herds in group 3 had an episode after 1998. Herd B had one standard reactor (a three-year-old cow) to the SICTT on February 25, 1999. Herd B was de-restricted with a clear six-month check on November 12, 1999, was clear at a round test on May 11, 2000, but was again deemed BTB-positive (after 533 days) on October 26, 2001 in a round test with one standard reactor (a steer, aged 1.5 years, born in this herd) that had a BTB lesion. Herd D disclosed an 'inconclusive' animal (a five-year-old cow) in August 7, 2001. This same cow was identified as a reactor on February 14, 2002. Thus two of the six group 3 herds may have had residual infection.

The remaining three herds with reactors were from group 1, one of them (herd five) had two standard reactors in 1998. The other two herds (herds two and 10) each had only one inconclusive animal in 1997 and 2000, respectively. Herd 10 broke down again in February 2002 and the previous inconclusive animal (cow, four years old) at this time was deemed a standard reactor. A final herd (herd three from group 1 herds; **Table 2**) had two BTB episodes during the fifth year, first in September 20, 2001 disclosing two standard reactors, both with lesions and subsequently on July 26, 2002 with three standard reactors.

Purchase of infected animals

Among the 31 BTB-positive herds, 16 of them were self-contained (the BTB-positive animals were born in that herd). The remaining 15 herds had BTB-positive animals that were not born in the herd. In cases in which the purchased animal passed at least one SICTT, we downplayed that animal as a potential source of infection (i.e., bought-in infection). Thus, two herds in group 2 purchased one animal each that reacted positively at the first SICTT in the 'new' herd (**Table 2**; herds 15 and 17). Herd 17 had no BTB confirmed at necropsy following the January 31, 2002 testing episode. None of the group 3 herds had reactors that had been purchased.

BTB status of neighbouring herds

As noted, group 1 herds had no neighbours with BTB during the fifth year of the FAP; however, almost all herds in group 2 had infected neighbours. Not surprisingly, because of selection criteria, the herds within group 3 had the higher proportion of neighbouring herds infected. The spatial pattern in group 3 (if not group 2) is consistent with over-the-fence spread as well as with a local ongoing source of infection such as infected badgers

*Badgers (*Meles meles*)*

The study area was under minimal badger removal activities. As part of national strategy to control BTB in cattle, routine ER76 investigations were conducted in herds having a BTB breakdown with multiple standard reactors. If the veterinary inspector identified that badgers were involved as a source of infection for cattle, a license was

granted to conduct 'reactive' badger culling in setts located in the land owned by the farmer. Licenses were granted to remove badgers from setts located in four different herds (labelled herds 14 and 19; X and C in **Table 2**). Herds 14 and 19 were immediate neighbours, as were herds X and C. In total, eight badger setts were visited in November 2002, and four badgers were caught from four different setts. None of these four badgers presented gross BTB lesions at post mortem examination. Only one of these badgers (25%) was classified by histopathology as *M. bovis* positive. The sett location from which this *M. bovis* positive badger was captured is shown in **Figure 3**.

Discussion

County Donegal is an area of Ireland that had low levels of BTB. Between 1989 and 1999, the APT (apparent prevalence for a thousand tested cattle) for Ireland varied from 1.5 (Co. Mayo) to 7.7 (Co. Monaghan) with a national average of 3.38. For Co. Donegal this value was 2.08 - the third lowest county (Byrne, 2000). The Co. Donegal removal and reference areas were selected for the FAP due to the presence of good natural geographical boundaries, its diverse Irish landscape and because the apparent disease prevalence for the period previous to the FAP study (1987-1995) was higher than in the rest of Co. Donegal (Centre for Veterinary Epidemiology and Risk Analysis, 2004).

The spatial pattern of BTB breakdowns in year five was different from previous years in that a group of neighboring herds in the eastern part of the study area were restricted at the same time. Within the cluster of groups 2 and 3 herds, one large herd (herd X) had numerous standard reactors and animals with BTB lesions found at slaughter. This was the most severe BTB breakdown during the entire study period. The data we have suggest that the infection in this herd was well established and perhaps of long duration. Herds in the vicinity of herd X also suffered BTB breakdowns of considerable severity, accounting for multiple standard reactors and animals disclosing BTB lesions. During the second year of the FAP a herd was also restricted with high number of standard reactors (n=12), but only two animals had BTB lesions. In this instance, only one neighbour was BTB-positive during the same year and, previously or subsequently, no major infection was found in the vicinity of this herd.

Herd size is known to influence the results of the SICTT because as the number of animals tested increases, the probability of finding at least one positive animal increases (O'Sullivan and O'Keefe, 1997). Griffin *et al.* (2005) reported that the average number of cattle per herd in reference area was 15; the average (and median) herd size of our subset of infected herds was two times (or 1.5 times) larger than the average herd in the county.

With respect to duration of infection, within a herd, BTB breakdowns with multiple standard reactors (or a higher animal-level BTB rate per animal-day at risk) suggest a greater infectivity, a management system that enhances spread of *M. bovis*, or a longer duration of the infection within the herd than in herds with smaller breakdowns. Similarly, in those herds in which BTB lesions were found at the slaughterhouse (and then confirmed *M. bovis* positive) it can be inferred that the infection was present for a longer period than in herds without

lesions, while bearing in mind the low sensitivity of lesion detection.

With respect to probable source of infection, purchase of infected animals occurred in two herds previous to their BTB breakdown during the fifth year within the cluster. However, since neither of these herds was linked directly to the core cluster of six infected herds it is thought that the purchase was an unlikely explanation for the cluster. In an earlier study, Olea-Popelka *et al.* (2004) demonstrated that herds that had a severe BTB breakdown (measured by the number of standard reactors) had a higher hazard, compared to less severe BTB breakdowns, of having a subsequent BTB breakdown. One of the conclusions in that study was that residual infection could explain this finding. In the current investigation, residual infection was possible since two or more episodes occurred in the same herd during the study period on 10 occasions (out of 72 herds). Of the 31 herds that developed BTB in the fifth year of the FAP, one herd (herd 3 in **Table 2**) had two different episodes during the fifth year, and five other herds had previous breakdowns in the past five years; two of these herds were in the group 3 herds. Herd B (an immediate neighbour of herd X and with the largest interval between tests) had a previous breakdown in 1999, disclosing one standard reactor with a BTB lesion. The animal that was identified as standard reactor with a BTB lesion on October 26, 2001 (during the fifth year of the FAP) was born in the herd and at the time of the previous herd test (May 11, 2000) was only one-month-old. The possibility that at the test on May 11, 2000 some infected animals in this herd failed to react to the SICTT (Monaghan *et al.*, 1994), and were the source of the infection for the animal subsequently identified on October 26, 2001, cannot be dismissed. In addition, another herd X neighbour (herd D) had an animal showing some evidence of infection since 2001 (an inconclusive reactor in 2001); this same cow was deemed a reactor in February 2002 during a contiguous SICTT test scheduled one month after another neighbour (herd C) had been restricted with a severe breakdown (**Figure 4** and **Table 2**).

As stated in the results section, herd X was tested eight and a half months after one of its neighbours (herd A) had a BTB breakdown. Even though the contiguous herd test for herd X was scheduled for November 17, 2001, it was not carried out until April 26, 2002. The delay in having this contiguous test carried out was because the farmer postponed his test until his 365-day trading window had come to an end. In Ireland, once a herd passes the annual screening test for TB (the round test), cattle from that herd may be sold, without further testing, for 365 days. The authors believe that this aspect of the BTB testing scheme deserves some critical re-assessment, especially in those scenarios in which epidemiological evidence suggests that a serious problem could be underway.

The analysis indicated that 61% of the breakdowns during the fifth year were comprised of epidemiologically related infected herds. Thus, the increased number of BTB breakdowns during the fifth year may have occurred because of herd-to-herd transmission, following recrudescence of earlier infection in some of the herds. Certainly, based on the number of standard reactors and animals found with BTB lesions at slaughter, herds X, C and E were likely to have had the infection for a relatively long time, and could have served as a source

of *M. bovis* to neighboring herds. In addition, herd B had a standard reactor in 1999. The ER76 reports confirmed that herd X and its neighbours had management practices that made over the fence ('nose to nose') contact possible between animals of different herds.

The mechanisms and factors that determine if *M. bovis* can initiate infection in a susceptible animal are not completely understood, but have been previously studied in detail by different authors (Smith, 1905; Garner, 1946; Francis, 1947; Rempt, 1954; Langmuir, 1961; McIlroy *et al.*, 1986; Neil *et al.*, 1988, 1989, 1991; Pollock *et al.*, 1993; Flanagan and Kelly, 1996; Costello *et al.*, 1998b; Philips *et al.*, 2003). There is agreement that only certain tuberculous individuals act as effective disseminators and they do so probably intermittently and only under certain circumstances (Morris *et al.*, 1994). Certainly, it is erroneous to assume that the only animals which excrete *M. bovis* are those with large clearly identifiable lung lesions (Morris *et al.*, 1994). Phillips *et al.* (2003) have summarised the different routes and mechanisms by which *M. bovis* could be transmitted to cattle.

In addition to herd-to-herd spread, the possible role of infected badgers cannot be disregarded. All the ER76 forms indicated that land fragments grazed by cattle (in herds with multiple 'standard reactors' during the fifth year) had evidence of badger activities. Four herds had active setts on their land and, in all the remaining farms, badger latrines or badger passes were identified and confirmed by a veterinary inspector. However, only four badgers were caught (from eight setts) and only one (25%) badger was diagnosed as *M. bovis* positive at histology examination. This level of infection does not differ from the 26.1% prevalence in badgers in the other three reference areas of the FAP. Thus, even though the role (infected) badgers can play in BTB in cattle (O'Connor and O'Malley, 1989; Dolan, 1993; Martin *et al.*, 1997; Olea-Popelka *et al.*, 2005; Griffin *et al.*, 2005) is recognised, the authors do not think the outbreaks during the fifth year should be attributed primarily to badgers. We have no evidence of excess TB in badgers, nor of a high density of badgers in this area. In such circumstances, transmission of BTB from a single, infected and highly-active, badger to a number of herds does not seem biologically-plausible.

Conclusion

Although the authors agree that infected badgers pose a serious risk to the general cattle population, in this instance it is difficult to point to badgers as the major cause of the elevated number of restricted herds during the fifth year. Given the history of the herds and the fact that residual infection could not be ruled out in two of the group 3 herds, the likely long duration of infection in some herds and that fence-line contact was deemed very feasible between herds, it would appear that herd-to-herd transmission could have been an important causal factor in the development of this cluster of infected herds. Purchase was not an explanation for infection in the group 3 herds. This study supports the hypothesis that BTB in herds is not a problem that can be addressed by dedicating our efforts to the elimination of single risk factors.

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