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1 **Tuberculosis in cattle herds are sentinels for *Mycobacterium bovis* infection**
2 **in European badgers (*Meles meles*): the Irish Greenfield Study**

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22 **Abstract**

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

45

46 *Keywords:* European badger; *Meles meles*; post mortem; tuberculosis; prevalence, sentinel
47 species.

48

49 **Introduction**

50 Tuberculosis (TB) is endemic in the Irish badger population (Dolan 1993) and badgers are a
51 source of infection for cattle (Griffin et al. 2005). Badgers are removed when an
52 epidemiological investigation associates a cattle herd TB breakdown with the presence of
53 badgers (focal culling) and is a medium-term strategy for the control of TB in cattle (O'Keeffe
54 2006). Studies using comprehensive post mortem examination and bacterial culture of tissues
55 have found an infection prevalence of 36-50% in culled badgers (Murphy et al. 2010); Corner
56 unpublished) and badgers culled in association with cattle herd breakdowns have a higher
57 prevalence of infection than the badger population at large (Corner et al. 2008). However, the
58 hypothesis that cattle can be used as sentinels for infection in the badger population has never
59 been formally examined. Badger culling is a medium-term control strategy pending the
60 development of a vaccine for badgers as a long-term control option. It is predicted that badger
61 vaccination will decrease the prevalence of infection in the population and facilitate
62 eradication of TB from livestock using currently available diagnostic procedures (Gormley
63 and Costello 2003). The development and evaluation of badger vaccination strategies would
64 be greatly enhanced by an understanding of the variation in infection prevalence in different
65 badger populations. The aim of the Greenfield study was to establish the infection prevalence
66 in badgers in areas of low prevalence of infection in cattle (Greenfield sites) and thereby test
67 the hypothesis that cattle can be used as sentinels for infection in associated badger
68 populations.

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73 **Materials and methods**74 **Selection of Greenfield sites**

75 The selection process identified badger setts in the areas of the country with the lowest cattle
76 TB herd prevalence over a 5 year period. The process utilised the Irish national bovine disease
77 database (Animal Health Computer System, AHCS), the Land Parcel Identification System
78 (LPIS) geographic database, and Geographical Information Systems (GIS) analytical
79 software. For the purposes of identifying suitable sites for the study, a map of Ireland was
80 overlaid with 112,440 hexagons with a cross-sectional width of 1 km (McGrath et al. 2008).
81 AHCS contains the locations of all cattle herds claiming area aid and are held in polygon
82 format on the LPIS spatial database. A unique herd identifier was used to join the spatial data
83 with the herd TB testing history.

84 Greenfield study sites were identified after all of the following areas (hexagons) were
85 excluded:

- 86 1. Those associated with herds that had a TB breakdown of >2 standard reactors in the 5
87 years, 2002 - 2006.
- 88 2. Those immediately adjacent to farms with ≤ 17 or ≥ 80 cattle (1st and 4th quartiles of
89 the population of cattle herds).
- 90 3. Those within 1 km of a badger sett culled under the national badger culling strategy.
- 91 4. Those touching any hexagons excluded using criteria 1 – 3.
- 92 5. Those overlaying commonage (common grazing land).
- 93 6. Very small 'island' Greenfield areas, i.e. areas consisting of 1 or 2 hexagons.

94 The internal hexagon boundaries of the remaining hexagons were then dissolved creating
95 Greenfield areas and the approximate centroid was assigned to each area. Each centroid was
96 then examined against a backdrop of geo-rectified orthophotography to ensure it was on or
97 close to agricultural land. Greenfield sites (198) were identified in 24 counties. When
98 surveyed, 138 sites had active badger setts or signs of badger activity. Trapping was
99 conducted at these sites using stopped restraints until one badger was captured or for a
100 maximum of 12 nights.

101 Fifteen months after the commencement of the study an investigation was undertaken of all
102 herds that had subsequently broken down and were within 500 metres of a capture site. A
103 distance of 500 metres is representative of the radius of the "average badger territory" within
104 the Irish context (G. McGrath, unpublished).

105 **Post mortem examination**

106 Captured badgers were anaesthetised with ketamine hydrochloride (0.1 ml/kg) and
107 medetomidine (Domitor[®]; 0.1 ml/kg), blood samples were collected by jugular venopuncture
108 and then the badgers were euthanased with an overdose of intravenous sodium
109 pentobarbitone.

110 The badgers were subjected to a detailed necropsy including the examination for bites or
111 wounds. The age (age classes: young < 18 months, adult or old, based on an assessment of
112 tooth wear), sex and weight of the badgers was recorded. Tissue samples for bacteriology
113 were collected using an aseptic technique to minimise contamination. The anatomical sites
114 examined for gross lesions and the specimens collected in 5 pooled samples for
115 bacteriological examination are listed in Table 1. Suspect gross lesions, including
116 wounds/bites, were collected separately. A sample (~2 g) was collected from a cross section
117 of each lobe and pooled for the lung sample. All samples were stored at -20⁰C prior to culture.

118 Bacterial culture

119 Tissue homogenates were decontaminated with 0.75% w/v cetylpyridinium chloride (CPC).
120 For primary isolation each sample was inoculated onto two plates of modified 7H11 medium
121 (Gallagher and Horwill 1977) containing a mixture of antibiotics (Polymyxin B 200,000
122 units/l, Ticarcillin 100 mg/l, Amphotericin B 10 mg/l and Trimethoprim 10 mg/l;
123 Mycobacteria selectatab*, Mast Diagnostics, Derby Road, Bootle, UK), two slopes of
124 Lowenstein Jensen medium with pyruvate (LJP), two slopes of Stonebrink's medium with
125 pyruvate (SB). Inoculated media were incubated at 37⁰C for 12 weeks with the exception of
126 the 7H11 plates which were incubated for 28 days. Preliminary isolate identification was
127 based on colony morphology, growth rate, pigmentation and cording characteristics and by
128 the AccuProbe DNA hybridisation probe for the *Mycobacterium tuberculosis* complex (Gen-
129 Probe, Inc., San Diego, Calif). Isolates were confirmed as *M. bovis* by spoligotyping, which
130 was performed according to the method of Kamerbeek et al. (1997).

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132 Cull badgers

133 A cull badger population was examined for comparative purposes. Cull badgers (n = 215)
134 were obtained from culling operations associated with cattle herd breakdowns and were
135 sourced from broad geographical areas across the Republic of Ireland (Murphy et al. 2010).
136 The median number of badgers captured was 2 per sett (range 1 - 7).

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146 **Results**

147 **Demographic data**

148 A badger was removed from 87 of the Greenfield sites (Figure 1). The age, sex and infection
149 status of the 87 badgers examined is shown in Table 2. More males (n = 47) than females (n =
150 40) were examined and more males were infected (19.1%, n = 9) than females (10%, n = 4).
151 A higher percentage of adult badgers (18.5%, n = 10) were infected than old (11.7%, n = 2) or
152 young (6.25%, n = 1) badgers. None of these differences were significant. There was no
153 significant difference in the proportion of infected animals with NVL or VL across age groups
154 or by sex.

155 **Nature and distribution of infection**

156 For the purpose of this study, a badger was considered infected when *M. bovis* was isolated by
157 bacterial culture from any sample. Using this standard, 13 of the 87 badgers examined were
158 infected, a prevalence of 14.9%. Of these 13 infected badgers 5 had gross visible lesions
159 confirmed by bacterial culture (VL), while the remaining 8 culture positive animals were
160 NVL. Generalised disease, defined as infection in all 5 pools, was encountered in only 1
161 badger and it was also NVL. The distribution of infected pools in infected badgers is shown in
162 Table 3. The thorax pool was affected in 61.5% (8/13) and the lungs in 23.1% (3/13) of
163 infected animals, but the lungs were never the sole site of infection. *M. bovis* was isolated
164 from 53.8% (7/13) of head pools and abdomen pools, and from 46.2% (6/13) of carcass pools
165 in infected badgers. There was no significant difference in the distribution of infection by
166 pool sample. The 6 infected animals that were positive in only one pool were all NVL.

167 During post mortem examinations 19 gross pathological lesions that were possibly
168 tuberculous were observed in 11 animals and of these, 12 lesions (63.2%), including three bite
169 wounds, were confirmed as tuberculosis (VL). The distribution of VL and other non-
170 tuberculous gross lesions is shown in Table 1. The 12 tuberculosis lesions were found in 5
171 animals. The VL were equally distributed across head, carcase and thoracic cavity (thorax
172 pool and lungs) pools, with no lesions found in the abdomen. Of the 7 non-tuberculous lesions
173 most (85.7%) were in the thoracic cavity. The 3 bite wounds (1 at the back of the knee, 1 on
174 the cheek and 1 at the axilla) were found in 2 (1.9%) badgers and *M. bovis* was isolated from
175 all three.

176 **Post sampling breakdowns**

177 There were 12 tuberculosis breakdowns (> 2 reactors) within 500m of the badger capture site
178 at 9 of the 87 Greenfield sites within 15 months of the commencement of the study. *M. bovis*
179 was isolated from 2 (22.2%) of the 9 badgers removed from these sites during the study.

180 **Comparison with cull badger population**

181 There was a significant difference ($P < 0.001$) in the prevalence of *M. bovis* infection in the
182 Greenfield study (14.9%) compared to that of the cull badger population (36.6%). However,
183 in the Greenfield study only 1 badger was removed per site, therefore when the cull badger
184 dataset is restricted to only badgers caught singly i.e. 1 badger removed per sett ($n = 77$), a
185 higher infection prevalence was seen (34 badgers, 44.2%).

186 In order to compare the distribution of infection in the Greenfield badgers to that in cull
187 badgers, it was necessary to combine the lungs and thorax pools to be comparable to the cull
188 badger study (Table 4). There was no significant difference in the distribution of infection in
189 the two studies. The mean number of culture positive pools was similar (Greenfield mean 2.2
190 and culled badgers mean 1.8; $P = 0.54$) and although badgers had more sites of infection in

191 the Greenfield study compared to the cull badgers study (46.2% with >2 positive pools
192 compared to 21.8%, Table 5.4) the difference was not significant ($P = 0.062$). There was a
193 higher percentage of VL badgers in the Greenfield study (38.5%) than in the cull badger study
194 (33.3%) although the difference was not significant ($P = 0.76$), and there was no significant
195 difference ($P = 0.11$) in the proportion of gross lesions that were confirmed as *M. bovis*
196 infection in the cull badger study (80%, 76/95; (Murphy et al. 2010) compared to the
197 Greenfield study (63%, 12/19). The distribution of gross tuberculous lesions was different in
198 the two studies. Greenfield badgers had a higher proportion of tuberculous bite wounds (25%
199 vs. 9.2%) and gross tuberculous lesions in carcase lymph nodes (25% vs. 11.8%) than cull
200 badgers, but Greenfield badgers had a lower proportion of thoracic lymph node (8.3% vs.
201 21.1%) and lung (16.7% vs. 28.9%) lesions than cull badgers.

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218 **Discussion**

219 The results of this study validate the use of cattle as sentinels for TB in badgers. An infection
220 prevalence of 14.9% was found in the badger population in areas where historically there was
221 a low prevalence of TB in cattle. The infection prevalence was significantly lower ($P <$
222 0.001) than that obtained in the cull badger study (36.3%; (Murphy et al. 2010). Intensive post
223 mortem procedures and bacteriology have been shown to yield more accurate prevalence
224 estimates in badgers (Crawshaw et al. 2008). The two studies are comparable as similar
225 detailed post mortem and bacterial culture techniques were used, except that a higher
226 concentration of decontaminant (0.75% vs. 0.075% CPC) was used in the Greenfield study.
227 Although the higher concentration of CPC, as with all decontaminants, will be more toxic for
228 *M. bovis* (Corner et al. 1995) and so effectively decreased the sensitivity of the culture
229 method in the Greenfield study, we do not believe that this invalidates the comparison of
230 results nor decreases the significance of the findings. There were differences in the selection
231 criteria used to identify Greenfield sites and cull sites but these only served to underestimate
232 the differences because the badgers examined in the cull badger study came from culling
233 operations that ranged from the first cull after a TB breakdown to the 4th annual repeat cull of
234 an area. By contrast, the Greenfield areas had never previously been treated. The prevalence
235 of infection has been shown to be higher in the first cull of a badger population than in
236 subsequent culls (Corner et al. 2008).

237 Confirmation of the hypothesis that cattle can be used as sentinels for infection in the badger
238 populations supports the current medium term national strategy for the control of bovine TB
239 in Ireland (O'Keeffe 2006). This strategy involves the focal removal of badgers where,
240 following an epidemiological investigation, they are identified as the probable source of a TB

241 outbreak in cattle. In the short term this results in a reduction in the local badger population, a
242 consequent reduction in badger-cattle interactions and a reduced risk to the index herd and
243 also to the surrounding herds (Olea-Popelka et al. 2009).

244 While this study has demonstrated that the prevalence of infection in badgers in areas of the
245 country with the lowest prevalence of infection in cattle is significantly lower than in bovine
246 TB “hot spot” areas, there were infected badgers within these Greenfield areas and yet, **at the**
247 **time of removal, they did not appear to have been causing significant breakdowns in local**
248 **cattle herds i.e. > 2 reactors.** The distribution of infection in the Greenfield badgers showed a
249 similar pattern to that in the cull badgers, with *M. bovis* isolated more frequently from the
250 thoracic cavity than other pools. There were, however, fewer gross tuberculous lesions in the
251 thoracic cavity of Greenfield badgers than focal cull badgers and, if this is a generalised
252 feature of lower prevalence areas, it could result in differences in the risk of transmission of
253 infection both between badgers and from badgers to cattle, and hence differences in infection
254 prevalence in the two populations. Alternatively, the reasons for the difference in infection
255 prevalence between the two populations may lie elsewhere. It may lie in differing
256 characteristic of the badger populations in these areas compared to in hot spot areas, leading
257 to differences in badger TB prevalence and the risk of transmission to cattle. The Greenfield
258 areas may have had lower badger densities than other areas. Lower badger densities could
259 result in lower contact rates between infected and susceptible badgers, and hence lower
260 transmission rates between badgers. A critical infection prevalence threshold may be required
261 in a badger population to facilitate the transmission to cattle. The Greenfield sites included
262 only farms with a herd size in the 2nd and 3rd quartile thereby excluding low cattle density as
263 an explanation of the lower risk of TB in cattle.

264 However, herd breakdowns did occur in these areas with historically low levels of TB. Within
265 15 months of commencement of the badger removals at Greenfield sites, 12 herds associated

266 with 9 (10%) of the 87 sites had a TB breakdown. Breakdowns in 4 herds at 3 sites were, after
267 an epidemiological investigation, deemed to be due to badgers. The selection criteria for
268 Greenfield sites was that only areas where herds had ≤ 2 reactors over a 5 year period were
269 included and so these were not TB free areas but rather areas with very low herd prevalence
270 and incidence. In developing the criteria for selection of Greenfield sites, we noted that for
271 every additional year that we examined over and above 5 years, Greenfield sites were lost. It
272 is possible that there is a cyclical nature to the status of Greenfield areas, with new
273 breakdowns occurring in areas with historically low TB herd incidence.

274 The finding of geographic variation in the prevalence of TB in Irish badger populations will
275 be used to devise practical badger vaccination strategies. This information will be used by
276 modellers and others to inform decisions regarding which badger populations to vaccinate and
277 how vaccine will be deployed. If the status of Greenfield areas is cyclical, a strategy of
278 identifying Greenfield areas on, for example, an annual basis and vaccinating badgers within
279 these areas, before the prevalence of infection in the badger and cattle populations increases,
280 could potentially break the badger-cattle cycle of disease. Following the present study an
281 additional one was undertaken to examine the prevalence of infection in badgers removed
282 from hot-spot areas but restricting the examination to badgers captured at the first cull after a
283 TB breakdown. The same post mortem and culture procedure were used in that study and the
284 results, when available, will allow a more accurate interpretation of the data from the
285 Greenfield study.

286

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293 operation with this study.

294

295 **Conflict of Interest Statement**

296 The authors declare that they have no financial and personal relationships with other people or
297 organisations that could inappropriately influence (bias) their work. Examples of potential
298 conflicts of interest include employment, consultancies, stock ownership, honoraria, paid
299 expert testimony, patent applications/registrations, and grants or other funding.

300

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349 **Table 1 The pooled samples collected for bacteriology, the tissues examined at**
 350 **post mortem for gross lesions, and the number of visible tuberculous lesions and**
 351 **non-tuberculous lesions confirmed by bacterial culture found in each tissue.**

Bacteriology pool	Tissue examined	Gross lesions	
		TB	Non TB
Head	Right parotid lymph node	1	0
	Left parotid lymph node	0	0
	Right mandibular lymph node	1	0
	Left mandibular lymph node	1	0
	Right retropharyngeal lymph node	0	0
	Left retropharyngeal lymph node	0	0
		25.0%*	
Carcase	Right prescapular lymph node	0	0
	Left prescapular lymph node	1	0
	Right axillary lymph node	1	0
	Left axillary lymph node	0	0
	Right inguinal lymph node	0	0
	Left inguinal lymph node	0	0
	Right popliteal lymph node	0	1
	Left popliteal lymph node	1	0
	25.0%*		
Abdomen	Spleen	0	0
	Liver	0	0
	Hepatic lymph node	0	0
	Mesenteric lymph node	0	0
	0.0%*		
Thorax	Anterior mediastinal lymph node	0	0
	Posterior mediastinal lymph node	0	1
	Right bronchial lymph node	1	0
	Left bronchial lymph node	0	2
	8.3%*		

Lungs	Left cranial lobe	1	0
	Left caudal lobe	0	0
	Right cranial lobe	1	0
	Right middle lobe	0	0
	Right caudal lobe	0	3
	Accessory lobe	0	0
		16.7%*	
Skin	Bite wound/ subcutaneous abscess	3	0
		25.0%*	
Total		12	7

352 * Indicates the percentage of visible tuberculous lesions found in each pool

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354 **Table 2 Age, sex and infection status of the 87 badgers examined**

Sex	*Age classes						Total		
	Adult		Old		Young		I	NI	
	I	NI	I	NI	I	NI			
Male	8	26	0	3	1	9	9	38	47
Female	2	18	2	12	0	6	4	36	40
Subtotal	10	44	2	15	1	15	13	74	
Total	54		17		16		87		

355 I = Infected

356 NI = non-infected

357 *Age classes determined by teeth wear

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374 **Table 3 Anatomical distribution of *M. bovis* infection in Greenfield badgers**

No. of pools positive	Head	Carcase	Abdomen	Thorax	Lungs	No. of animals infected
1	2	1	1	2	0	6
2	0	0	0	1	1	1
3	1	2	2	1	0	2
4	3	2	3	3	1	3
5	1	1	1	1	1	1
Total	7	6	7	8	3	13

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Table 4 Anatomical distribution of infection in *M. bovis* culture positive animals in (a) the Greenfield study and (b) the cull

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badger study

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	No. pools positive	Head	Carcase	Abdomen	Thorax & Lungs*	No. of animals infected
(a)	1	2	1	1	3	7
	2	0	0	0	0	0
	3	2	2	3	2	3
	4	3	3	3	3	3
	Total	7	6	7	8	13
(b)	1	12	9	2	14	37
	2	13	12	6	17	24
	3	6	10	8	12	12
	4	5	5	5	5	5
	Total	36	36	21	48	78

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* Thorax pool and lungs combined

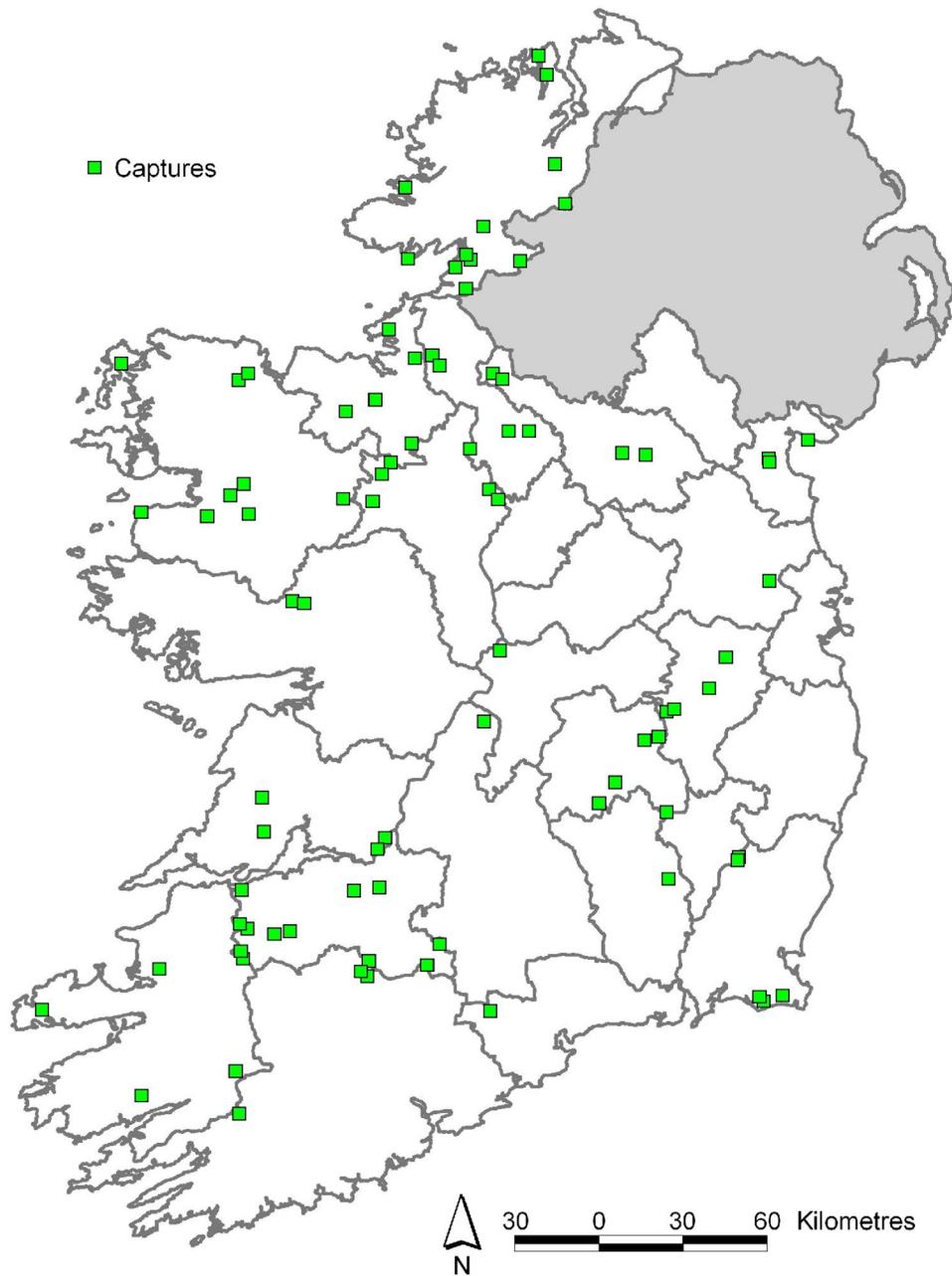
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410 **Figure 1** The locations of the 87 Greenfield sites where a badger was
411 captured



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