



HAL
open science

Trial Design to Estimate the Effect of Vaccination on Tuberculosis Incidence in Badgers

Inma Aznar, Guy Mcgrath, Denise Murphy, Leigh A.L. Corner, Eamonn Gormley, Klaas Frankena, Simon J. More, Wayne Martin, James O’Keeffe,
Mart C.M. de Jong

► **To cite this version:**

Inma Aznar, Guy Mcgrath, Denise Murphy, Leigh A.L. Corner, Eamonn Gormley, et al.. Trial Design to Estimate the Effect of Vaccination on Tuberculosis Incidence in Badgers. *Veterinary Microbiology*, 2011, 151 (1-2), pp.104. 10.1016/j.vetmic.2011.02.032 . hal-00701908

HAL Id: hal-00701908

<https://hal.science/hal-00701908>

Submitted on 28 May 2012

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L’archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d’enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

Accepted Manuscript

Title: Trial Design to Estimate the Effect of Vaccination on Tuberculosis Incidence in Badgers

Authors: Inma Aznar, Guy McGrath, Denise Murphy, Leigh A.L. Corner, Eamonn Gormley, Klaas Frankena, Simon J. More, Wayne Martin, James O’Keeffe, Mart C.M. De Jong



PII: S0378-1135(11)00113-1
DOI: doi:10.1016/j.vetmic.2011.02.032
Reference: VETMIC 5199

To appear in: *VETMIC*

Please cite this article as: Aznar, I., McGrath, G., Murphy, D., Corner, L.A.L., Gormley, E., Frankena, K., More, S.J., Martin, W., O’Keeffe, J., De Jong, M.C.M., Trial Design to Estimate the Effect of Vaccination on Tuberculosis Incidence in Badgers, *Veterinary Microbiology* (2010), doi:10.1016/j.vetmic.2011.02.032

This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

1 **Trial Design to Estimate the Effect of Vaccination on Tuberculosis Incidence in Badgers**

2

3 **Inma Aznar**^{1*}, Guy McGrath¹, Denise Murphy², Leigh A.L. Corner³, Eamonn Gormley³,
4 Klaas Frankena⁴, Simon J. More¹, Wayne Martin⁵, James O’Keeffe², Mart C.M. De Jong⁴

5

6 ¹ *CVERA, Veterinary Science Centre, School of Agriculture Food Science and Veterinary*
7 *Medicine, University College Dublin, Belfield, Dublin 4, Ireland*

8 ² *Department of Agriculture, Fisheries and Food (DAFF), Ireland*

9 ³ *Veterinary Science Centre, School of Agriculture Food Science and Veterinary Medicine,*
10 *University College Dublin, Belfield, Dublin 4, Ireland*

11 ⁴ *Quantitative Veterinary Epidemiology group, Wageningen Institute of Animal Sciences,*
12 *Wageningen University, P.O. Box 338, 6700 AH Wageningen, The Netherlands*

13 ⁵ *Department of Population Medicine, University of Guelph, Guelph, ON, Canada N1G2W1*

14

15 * inma.aznar@ucd.ie

16 **1 Abstract**

17

18 The principal wildlife reservoir of *Mycobacterium bovis* in Ireland is the European badger.
19 Studies in the Republic of Ireland (RoI) have shown that badgers culled in association with cattle
20 herd tuberculosis (TB) breakdowns (focal culling) have a higher prevalence of infection than the
21 badger population at large. This observation is one rationale for the medium term national
22 strategy of focal badger culling. A vaccination strategy for the control of TB in badgers is a
23 preferred long-term option. The Bacillus Calmette-Guérin (BCG) vaccine has been shown to
24 decrease disease severity in captive badgers under controlled conditions. As the vaccine has been
25 tested in a controlled environment with precise information on infection pressure, it cannot be
26 assumed *a priori* that the effects of vaccination are similar in the wild, where other environmental

27 and/or ecological factors prevail. For this reason we have designed a vaccine field trial to assess
28 the impact of vaccination on the incidence of TB infection in a wild badger population.

29

30 The selected study area for the vaccine trial (approximately 755 square kilometers) is divided into
31 three zones each of which has similar characteristics in terms of size, number of main badger
32 setts, cattle herds, cattle and land classification type. Three vaccination levels (100%, 50% and
33 0%) will be allocated to the three zones in a way that a gradient of vaccination coverage North to
34 South is achieved. The middle zone (Zone B) will be vaccinated at a 50% coverage but Zone A
35 and C will be randomly allocated with 100% or 0% vaccination coverage. Vaccination within
36 Zone B will be done randomly at individual badger level.

37

38 The objective of this paper is to describe the design of a field TB vaccination trial for badgers, the
39 epidemiological methods that were used to design the trial and the subsequent data analysis. The
40 analysis will enable us to quantify the magnitude of the observed vaccination effect on *M. bovis*
41 transmission in badgers under field conditions and to improve our knowledge on the biological
42 effects of vaccination on susceptibility and infectiousness.

43

44 *Keywords:* Vaccine trial, badgers, BCG vaccine, Ireland, tuberculosis, *M. bovis*

45

46

47 **2 Introduction**

48

49 Ireland initiated an eradication program for bovine tuberculosis (bTB) as early as 1950 (More and
50 Good, 2006). The adopted test-and-slaughter policy achieved a 97% reduction in cattle
51 tuberculosis prevalence, from 17% to 0.5% prevalence in the initial ten years (Watchorn, 1965).

52 Since then, bTB prevalence has remained relatively unchanged despite the introduction of a range
53 of measures aimed at reducing cattle to cattle transmission (Griffin and Dolan, 1995).

54

55 In the 1970s in England, badgers were first suspected as a reservoir for *Mycobacterium bovis*
56 (Krebs, 1997). The first infected badger was discovered in Ireland in 1974 (Noonan et al., 1975).
57 Since then numerous papers have been published that confirm badgers as the main wildlife *M.*
58 *bovis* reservoir in Ireland and England (Barrow and Gallagher, 1981; Cheeseman et al., 1981;
59 Fagan, 1993; Gallagher et al., 1998). A recent study carried out in Ireland detected a prevalence
60 of 36.3% in badgers trapped as part of Department of Agriculture, Fisheries and Food (DAFF)
61 culling operations; the prevalence reported here was much higher than in previous studies where
62 less comprehensive bacteriological culture methods had been used (Murphy et al., 2010).

63

64 The precise role of badgers in the dynamics of bTB is not clear. Several studies in Ireland have
65 linked badger removal with a subsequent reduction in bTB incidence (Eves, 1999; Griffin et al.,
66 2005; More and Good, 2006; Olea-Popelka et al., 2009). However, in a field trial carried out in
67 Britain, the reduction in cattle TB incidence in culled areas was only modest and an increase in
68 TB incidence, albeit transient (Jenkins et al., 2008), was observed in non culled neighboring areas
69 (Woodroffe et al., 2007). Pope et al. (2007) concluded that the increased prevalence observed in
70 neighboring areas was associated with medium and long-distance badger dispersal and
71 emphasized the importance of taking into account the potential negative effects associated with
72 badger dispersal when using culling as a disease control strategy. Although there are
73 discrepancies between different studies about the efficiency of badger culling in the control of
74 bTB, they all provide compelling evidence that badgers play an important role in the maintenance
75 of it. Therefore, addressing infection in badgers is considered vital when trying to control bovine
76 tuberculosis in the aforementioned countries. In the short- to medium-term in Ireland, focused
77 badger culling is being conducted as part of a broader national TB control programme, to limit

78 TB maintenance in areas where TB incidence in cattle is high. However, badgers are a protective
79 species in the Republic of Ireland (ROI) under the 1976 Wildlife Act. Consequently, alternative
80 strategies to badger culling are being sought in the long term for the control of bovine
81 tuberculosis.

82

83 In 2001, a 10 year work program was designed in Ireland to study the possibility of using *Bacillus*
84 Calmette-Guérin (BCG) vaccine in badgers to assist in the control and eradication of TB in cattle.
85 The program consisted of a sequence of experimental studies carried out initially with captive
86 badgers (Corner et al., 2007; Lesellier et al., 2009). Although vaccines can be tested in a
87 controlled environment for evidence of both protection and decreased transmission, it cannot be
88 assumed *a priori* that the effects of vaccination are similar in the wild where other factors may
89 play a role. For this reason, a vaccine field trial to assess the impact of vaccination on the
90 incidence of tuberculosis in a wild badger population has been designed as part of the ten year
91 project.

92

93 The objective of this paper is to describe a field trial design, the epidemiological methods that
94 were used to design the trial and the subsequent data analysis. The analysis will enable us to
95 quantify the magnitude of the observed vaccination effect on transmission under field conditions
96 and to improve our knowledge on the biological effects of the vaccination on susceptibility and
97 infectiousness of badgers.

98

99 **3 Theoretical basis: a review**

100

101 *3.1 Initial considerations in designing a vaccine trial*

102

103 Specifying clear question(s) of interest is essential when designing a trial to evaluate the effects
104 of vaccination. Different vaccination programs have different aims; the question of interest could
105 vary, for example from how good vaccination is in protecting the individual against infection to
106 what reduction in infectiousness can be achieved. The effect of interest will determine the study
107 unit, parameters of effect, as well as the level of information required (Halloran et al., 1997). The
108 main question of interest in our study is to determine the efficiency of badger vaccination in
109 reducing *M. bovis* transmission. Specifically we are interested in the value of the reproduction
110 ratio (R) at different levels of vaccination (p); ie $R(p)$. We will clarify how to estimate $R(p)$
111 subsequently.

112

113 Vaccines that reduce transmission in a population can have a beneficial effect in vaccinated as
114 well as in unvaccinated individuals. Most studies of vaccine efficacy emphasize the direct benefit
115 of vaccination (often called ‘protective ability’) to vaccinated individuals. Following Halloran et
116 al. (1999), we denote this as VE_S or Vaccine Efficacy for Susceptibility. In addition, there often
117 are indirect benefits for both vaccinated and unvaccinated individuals which Halloran et al.
118 denoted as Vaccine Efficacy for Infectiousness (VE_I). These measures will be estimated in this
119 trial; however, for subsequent modelling purposes we will emphasize $R(p)$

120

121 3.2 *Parameters of effect*

122

123 In order to estimate $R(p)$, we define four transmission parameters:

- 124 • transmission from a vaccinated to a vaccinated badger (β_{VV});
- 125 • transmission from a vaccinated to an unvaccinated badger (β_{VU});
- 126 • transmission from an unvaccinated to a vaccinated (β_{UV}), and
- 127 • transmission from an unvaccinated to an unvaccinated badger (β_{UU}).

128

129 The average number of secondary cases caused by one typically infected individual in a fully
130 susceptible population (Diekmann and Heesterbeek 2000) is called the Basic Reproduction Ratio
131 (R_0). Reproduction ratios of host populations in which interventions are taking place are often
132 represented by R to distinguish them from R_0 . The reproduction ratio as a function of the
133 proportion of vaccinated badgers $R(p)$ can be determined from the transmission rates described
134 above and the time that infected badgers remain infectious (T). Transmission rates are a
135 combination of the infectiousness of the donor and the susceptibility of recipient individuals, and
136 since vaccination with BCG has the potential to affect both of these, $R(p)$ will be an important
137 parameter for understanding the impact of badger vaccination in disease transmission and
138 population dynamics of *M. bovis*. Ultimately, the $R(p)$ obtained from the badger vaccine trial will
139 be used in further modelling aimed at assessing badger vaccination as a strategy to
140 control/eradicate *M. bovis* infection in cattle.

141

142 As noted above, vaccine efficacy/effectiveness has been traditionally defined as 1 minus some
143 measure of relative risk (RR) of the incidence of disease in the vaccinated group compared to the
144 incidence in the non-vaccinated group (Halloran et al., 1999). In the past, the main objective of
145 human vaccine studies was to measure individual protection against infection or disease (VE_S).
146 Perhaps less appreciated, but not less important, is the ability of a vaccine to reduce the duration
147 or severity of the infectiousness of those vaccinates that become infected (VE_I) (Longini et al,
148 1998). The latter effect has been reported in vaccine studies using BCG vaccine by the
149 subcutaneous or mucosal routes in badgers (Corner et al., 2008). In these experiments, *M. bovis*
150 was recovered from both vaccinated and non-vaccinated badgers after being challenged with the
151 mycobacterium; however, a reduction in the size, number and distribution of gross and
152 histological lesions in vaccinated badgers compared to non-vaccinated badgers was demonstrated.
153 Vaccination did not confer individual protection against infection in the mentioned study, but this

154 has to be cautiously interpreted as vaccine protection could be dependent on the infection dose. It
155 is not known what the infectious dose is in natural infections. In field vaccine trials in possum in
156 New Zealand, using conjunctival and intranasal vaccination (Corner et al., 2002) and more
157 recently vaccination by oral delivery (Tompkins et al., 2009), significant protection against
158 natural exposure was seen in the vaccinated group compared to the control groups. Protection was
159 much higher than predicted from previous studies where possums had been experimentally
160 challenged (Corner et al., 2001; Buddle et al., 2006).
161 Estimation of $R(p)$, VE_S and VE_I will give us a more detailed understanding of the ways BCG
162 vaccine works in a wild badger population.

163

164 **4 Epidemiological contribution to the design of the vaccine trial**

165 *4.1 Study site*

166

167 Prior to deciding on our study site for the badger vaccine trial, several epidemiological and
168 logistic factors were considered:

- 169 • *Culling history.* Because of the badger culling policy in Ireland, it was important to
170 have confidence that the area selected for the trial had been protected from culling
171 for some time before the commencement of the trial. Badger culling in that area
172 could have had a negative effect not just in the total number of individuals captured
173 but also on the initial prevalence of TB among badgers.
- 174 • *Knowledge of sett location.* Knowledge of the area in terms of sett location was
175 considered an advantage and helped in dividing the study area into three similar
176 zones (see below).

- 177 • *Community and technical support.* Good support from the local farming community
178 as well as from from both the District Veterinary Offices (DVOs) and Regional
179 Veterinary Laboratories (RVLs) was vital.

180 Based on these criteria, the area selected for the vaccine trial is located in County Kilkenny
181 (Figure 1). The size of the area is approximately 755 square kilometers. This area had been part
182 of one of the reference areas in the Four Area Project (FAP) (Griffin et al., 2005) and will have
183 been protected from culling for at least two years before the start of the vaccine trial. A
184 prevalence of infection of 30% is expected based on historical data from neighbouring areas.

185

186 4.2 *Trial design*

187

188 A vaccine trial to exclusively determine VE_S can be designed by vaccinating one or several
189 populations with the same vaccination coverage, with coverage being strictly inferior to 100%
190 (40%, 50%, 60%, etc) so that a number of infections within the vaccinated group can occur.
191 Because we aimed to estimate both VE_S and VE_I , then two populations vaccinated with different
192 vaccination coverage are required (Longini et al., 1998). In this paper it is explained how the
193 vaccination fractions for these two populations have to be selected to maximise the differences on
194 indirect effects between the two populations. This can be achieved with one population
195 vaccinated at 0% and the other vaccinated at the highest percentage that allows a minimum
196 number of infections to happen (critical vaccination fraction). Likewise to estimate the four betas
197 (transmission rates), different levels of coverage are needed (DeJong et al., 1996).

198

199 Our objectives could have therefore been attained with two populations, one vaccinated at 0%
200 and the other at 100% vaccination coverage (badger trapping rates will never be 100% effective;
201 also the dynamics of the badger population ensures a certain number of susceptible badgers every

202 year and therefore a certain number of infections). We chose though to include a third population
203 vaccinated at 50% to optimise the design in two ways: firstly by making sure that there will be
204 enough new infections even if the "100%" vaccination would lead to $R < 1$ and secondly by
205 allowing us to estimate the area effect (in the case of only two populations, the parameters would
206 be estimated from different populations and it would not be possible to disentangle the area
207 effect).

208

209 The trial area is divided into three zones with similar characteristics in terms of size, number of
210 main badger setts, cattle herds, cattle and land classification (Figure 2). In these zones,
211 vaccination coverage will be of allocated 100, 50 and 0%, with the gradient of coverage (either
212 100% to 0% from north to south, or vice-versa) being allocated randomly at the start of the trial.
213 Badger data were acquired through rigorous surveying of the entire study area. Previous
214 surveying had been performed in sections of the study area as part of the FAP reference area and
215 through the activities of the DAFF's licensed badger culling policy on setts adjacent to herd bTB
216 breakdowns. Setts previously recorded were revisited during the recent survey and assessed for
217 signs of activity. All bovine data were derived from the Animal Health Computer System
218 (AHCS) and farm outlines were taken from the Land Parcel Identification System (LPIS). Land
219 use classifications were defined using the CORINE dataset (Coordination of Information on the
220 Environment, 2000). Using ArcMap 9.2 (ESRI, Redlands, CA, USA) with geo-rectified colour
221 orthophotography and vectorized 1:50,000 data (Ordnance Survey Ireland, Dublin, Ireland),
222 natural boundaries, where possible, were selected to define the perimeter and internal boundaries
223 of the study area. The aim was to achieve similar infection pressure from cattle and badgers in the
224 three zones.

225

226 Badgers will be captured, treated with a vaccine or a placebo depending on the randomly
227 allocated treatment and then released. In zone B (with 50% vaccine coverage), each badger at

228 first capture will be randomly allocated to either vaccine or placebo. In each zone throughout the
229 study period, the treatment will be administered every year to avoid possible waning of vaccine
230 effects. Live *M. bovis* BCG strain Danish will be used. It will be prepared in a lipid formulation
231 for oral delivery, containing 10^8 colony forming units/ml. A lipid-only placebo with identical
232 visual characteristics, texture and viscosity to the vaccine, and in identical syringes, will also be
233 used (Aldwell et al., 2003a; Aldwell et al., 2003b). Vaccine and placebo control samples will be
234 coded at the laboratory where they are prepared, and neither field staff nor data analysts will be
235 aware of the vaccine status of individual animals.

236

237 The trial will employ a capture-tag-release regime with both cages and stopped wire restraints
238 being used. Badgers in the trial will be 'hand vaccinated'; that is, each animal will be individually
239 orally dosed with the vaccine. Each badger will be permanently identified with a tattoo and
240 passive transponder (microchip) when first captured. At each capture, badgers will be examined
241 and a blood sample collected. Humoral immune responses (serologic titres) will be used to
242 determine the badger's infection status and to detect a change in infection status; that is, to detect
243 both pre-existing infection in badgers as they are recruited to the study and the occurrence of new
244 infections on recapture. Key data, including sex, estimated age (cub, juvenile, and yearling, adult
245 and old adult), body weight, presence of injuries and the GPS location of the cage trap or
246 restraint, will be recorded at each badger capture. All data collected in the field will be recorded
247 onto handheld computers. The trial will last four years and there will be two 'catching' sweeps of
248 the entire area each year. At the end of the trial, badgers in the three zones will be depopulated
249 and a detailed post mortem examination will be conducted on all badgers, involving an
250 examination for gross pathology and the collection of samples for histopathology and
251 bacteriology. The severity of infection will be assessed from the number, distribution and the
252 severity of gross lesions, the number and distribution of histological lesions, and the number and
253 distribution of culture positive tissues and the bacterial load in those tissues.

254

255 Badger removal will be undertaken within the trial area when three or more standard reactors are
256 disclosed in a herd only if active badger setts are found in the farm and all other sources of
257 infection (residual, purchase and farm to farm spread) have been ruled out by an epidemiological
258 investigation. If culling of badgers is deemed necessary for control of tuberculosis in cattle herds,
259 the culling will be carried out by field staff of the project when they next trap in the designated
260 area.

261

262 **5 Analysis of the vaccine trial data**

263

264 *5.1 General description*

265

266 The outcome from the vaccine trial will be in the form of a Bernoulli experiment: as badgers are
267 trapped and a blood sample taken, we will gather information on whether these badgers have
268 become or “are” infected (assigned value 1) or not (assigned value 0) during the time at risk (time
269 between two subsequent trappings). Infection in this case will be defined by serologic results i.e.
270 by sero-positivity. Records on individual badgers will be taken at each successful capture (not
271 necessarily at each trapping exercise) such as location of the badger at the time of the trapping
272 (zone A, B or C) and its vaccination status. Other demographic data will be also recorded.

273

274 From the observations at each subsequent capture of each individual badger, the following
275 variables will be extracted:

276

- 277 • Time interval (Δt) between the two captures

- 278 • Disease status of the badger at the beginning and at the end of the time interval
- 279 • Vaccination status of the badger
- 280 • Zone where the animal was observed at the beginning and end of the time interval (A, B
- 281 or C)
- 282 • Average prevalence of infection in the zone where the badger was caught during Δt
- 283 (PrevA, PrevB, PrevC)
- 284 • Average fraction of infected badgers that are vaccinated during Δt in the zone where the
- 285 badger was caught (F_{VA} , F_{VB} , F_{VC})

286

287 The rate at which vaccinated/non-vaccinated badgers acquire infection from vaccinated and non-
 288 vaccinated badgers (β_{VV} , β_{VU} , β_{UV} , β_{UU}) can be estimated based on the observed probability of
 289 becoming infected for each of these individuals. The estimated β s will be used to calculate VE_s ,
 290 VE_I and the reproduction ratio as a function of the vaccination coverage ($R(p)$).

291

292 5.2 Estimation of the transmission parameters

293

294 The rate at which vaccinated/non-vaccinated badgers acquire infection from vaccinated and non-
 295 vaccinated badgers (β_{VV} , β_{VU} , β_{UV} , β_{UU}) can be estimated based on the observed probability of
 296 becoming infected for each of these individuals. For the purposes of explanation, we shall at first
 297 ignore the vaccination state of the badgers. A stochastic susceptible-infectious (SI) model can be
 298 used then to describe the transmission of *M. bovis* in the trial where transmission can occur if an
 299 infectious and a susceptible individual make contact. The number of infectious contacts (NIC,
 300 contacts with an infectious individual) encountered by each randomly chosen susceptible
 301 individual in a period of time Δt can be expressed as:

302

$$303 \quad NIC = \beta * (I / N) * \Delta t$$

304 Where:

305

306 β = Transmission parameter

307 Δt = Time interval

308 I= Number of infected individuals*

309 N= Total number of individuals

310 I/N= Prevalence of infected badgers*

311 * For our purpose infected badgers are deemed to be infectious

312

313 From the above it can be derived that the number of ‘successful’ infectious contacts (infectious
314 contacts that result in transmission events) encountered by this randomly chosen individual in a
315 period of time Δt follows a Bernoulli distribution with probability:

316

$$317 \quad p = 1 - e^{-\beta * I * \Delta t / N}$$

318

319 The observed number of new infections (C) among all susceptible badgers at the end of the time
320 interval between two captures can then be modelled using a binomial distribution where S is the
321 number of susceptible badgers at the beginning of the time interval and the probability that each
322 of these badgers will become infected during that time interval is defined by $1 - e^{-\beta * I * \Delta t / N}$

323

$$324 \quad E(C) = S * (1 - e^{-\beta * I * \Delta t / N})$$

325

326 Based on serologic results we will be able to determine the number of new cases among the
 327 susceptible badgers (C). By using a Generalized Linear Model (GLM) with a complementary-log-
 328 log link function, a binomial error function, with binomial total S and offset $\ln((I/N) * \Delta t)$, the
 329 transmission parameter β can be estimated.

330

331 Following the introduction of the vaccination protocol, there will be heterogeneity in the
 332 vaccination status of badgers; we will have four different β values with two sub indexes, of the
 333 type β_{vu} . The first sub index will refer to the vaccination state of the infectious badger and the
 334 second sub index to the vaccination state of the susceptible animal (to which the first has made
 335 contact). Clearly, we know the vaccination status of the receiving susceptible badger and thus we
 336 can calculate β_{vu} and β_{uv} separately. However to account for donor (infectious) badger, we use the
 337 differences in the fraction of infected badgers that are vaccinated in the different zones.

338

339 Let's assume that a badger that was originally trapped in zone A at time 't' is trapped again in
 340 zone A at time 't+1' and that this badger had been vaccinated. Vaccination coverage in zone A is
 341 targeted to be 100%, but since not all badgers will be trapped in each trapping exercise it will take
 342 2-3 trapping rounds to approach this goal. Our hypothetical badger will then have infection
 343 pressure coming from both vaccinated and non-vaccinated infected badgers. The number of
 344 infectious contacts encountered by this randomly selected badger in that period of time is defined
 345 by:

346

$$347 \quad NIC = (\beta_{VV} * I_V / N + \beta_{UV} * I_U / N) * \Delta t$$

348

349 If we assume that there is a multiplicative effect on *NIC* as the fraction of infected vaccinated
 350 badgers increases, after doing some algebraic manipulations (De Jong et al., 1996):

351

352

353

$$NIC = e^{\beta_{VV} * F_{VA} + \beta_{UV} * (1 - F_{VA})} * PrevA * \Delta t$$

354 or:

355

356

$$NIC = e^{\beta_{UV} + (\beta_{VV} - \beta_{UV}) * F_{VA}} * PrevA * \Delta t$$

357

358 where F_{VA} is the fraction of infected badgers that are vaccinated in zone A and $PrevA$ is the359 average prevalence of infection in zone A during Δt . If we set $\beta_{UV} = K_0$ and $(\beta_{VV} - \beta_{UV}) = K_1$ then

360 we can write:

361

362

$$E(C_V) = S * (1 - e^{-e^{(K_0 + K_1 * F_{VA})} * PrevA * \Delta t})$$

363

364 Since we will know the observed infection status of the specific badger (C_V) at the end of the time365 interval (Δt), we can fit a GLM with $\text{Log}(PrevA \Delta t)$ as an offset and calculate K_0 and K_1 . We can366 subsequently calculate β_{UV} and β_{VV} as:

367

368
$$\beta_{UV} = \text{Exp}[K_0]$$

369
$$\beta_{VV} = \text{Exp}[K_0 + K_1]$$

370

371 If we apply the same logic to model the observed infectious status of an unvaccinated badger (C_U)372 that was trapped in zone A at time t and at time $t+1$, we could estimate β_{UU} and β_{VU} as:

373

374

$$E(C_U) = S * (1 - e^{-e^{(k_0 + k_1 * F_{VA})} * PrevA * \Delta t})$$

375

376 where $\beta_{UU} = \text{Exp}[k_0]$ and $\beta_{VU} = \text{Exp}[k_0 + k_1]$

377

378 This way we can estimate the four betas based on incidence data from all three zones. In zone C

379 there will be no (or very few) vaccinated badgers so F_{VC} will be zero and that zone does not380 contribute to the estimation of k_1 and thus that area does not give information for β_{VU} .

381

382 Vaccine Efficacy for Susceptibility can be calculated then as:

383

384
$$VE_S = 1 - (\beta_{UV} / \beta_{UU})$$

385

386 We can also calculate Vaccine Efficacy for Infectiousness as:

387

388
$$VE_I = 1 - (\beta_{VU} / \beta_{UU})$$

389

390 Finally we can estimate $R(p)$ where p is the proportion of vaccinated as:

391

392
$$R(p) = \frac{1}{2} * [(1-p) * \beta_{UU} + p * \beta_{VV} + \sqrt{((1-p) * \beta_{UU} + p * \beta_{VV})^2 + 4 * p * (1-p) * \beta_{UV} * \beta_{VU}}] * T$$

393

394

395 where T is the duration of the "infectious" period of a typical infected individual (for relative396 comparisons of $R(p)$ knowledge of T is not essential).

397 For decision making, if $p=0$ and $\beta_{UU}T < 1$, then no vaccination is needed; if $p=1$ and $\beta_{VV}T > 1$,
398 vaccination will not stop spread of the disease. Otherwise, the vaccination fraction necessary in
399 order to achieve $R(p) < 1$ can be calculated.

400

401 **6 Concluding remarks**

402

403 The aim of this paper was to present the theory applied to the design of a badger vaccine trial, as
404 well as the epidemiological methodology and statistical analysis that will help to interpret the
405 results obtained.

406

407 The vaccine trial area was divided into three zones A, B and C, such that differences in infection
408 pressure in the three zones at the beginning of the trial will be minimized. However, there is an
409 inherent assumption that the contact patterns between badgers will be similar in the three zones.
410 We believe that this is a reasonable assumption as the size of the total area is sufficiently small,
411 and the landscape and distribution of setts is very similar in the three zones. Further, changes with
412 respect to infection pressure in the three zones will occur as the vaccine starts working. Our
413 model is designed to adjust for these changes. For simplicity, we have only presented the analysis
414 relevant to badgers repeated trapped in the same zone. However, the final model can be modified
415 to accommodate other scenarios where badgers move from one zone to another.

416

417 Where possible, natural boundaries are used to define the perimeter of the study area. The internal
418 boundaries between the three zones include streams, rivers and roads. The external boundaries,
419 while not bio-secure, should be substantial enough to delineate badger territories. This would
420 reduce the extent of typical badger movement into and out of the study area.

421

422 Defining specific questions of interest in the early stages of the vaccine trial was considered
423 crucial. Estimation of the reproduction ratio ($R(p)$) calculated as a function of the vaccination
424 coverage (p) will give us invaluable information on the impact of vaccination in disease
425 transmission and dynamics of *M. bovis* infection in badgers. The importance of considering the
426 potential indirect effects associated with the vaccination program in badgers has been highlighted
427 in the paper; furthermore, it has been shown how to estimate $R(p)$, VE_S and VE_I by using
428 different vaccination coverages in the study area.

429

430 Although the vaccine trial will not determine whether bTB can be eradicated in cattle and badgers
431 through a strategy of badger vaccination, the parameters obtained in the vaccine trial will be used
432 in a mathematical model of bTB transmission (currently under development) in order to assess
433 different control and eradication options for bTB in cattle in the Republic of Ireland.

434

435 **7 Conflict of Interest Statement**

436

437 The authors have not declared any conflict of interest.

438

439 **8 References**

440 Aldwell, F. E., Keen, D.L., Parlane, N.A., Skinner, M.A., de Lisle, G.W., Buddle, B.M., 2003a.

441 Oral vaccination with *Mycobacterium bovis* BCG in a lipid formulation induces
442 resistance to pulmonary tuberculosis in brushtail possums. *Vaccine* 22, 70-76.

443 Aldwell, F.E., Tucker, I.G., de Lisle, G.W., Buddle, B.M., 2003b. Oral delivery of

444 *Mycobacterium bovis* BCG in a lipid formulation induces resistance to pulmonary
445 tuberculosis in mice. *Infection and Immunity* 71, 101-108.

- 446 Barrow, P.A., Gallagher, J., 1981. Aspects of the epidemiology of bovine tuberculosis in badgers
447 and cattle. I. The prevalence of infection in two wild animal populations in south-west
448 England. *J. Hyg. (Lond)* 86, 237-245.
- 449 Buddle, B.M., Aldwell, F.E., Kee, D.L., Parlane, N.A., Hamel, K.L., de Lisle, G.W., 2006. Oral
450 vaccination of brushtail possums with BCG: investigation into factors that may influence
451 vaccine efficacy and determination of duration of protection. *N.Z. Vet. J.* 54, 224-230.
- 452 Cheeseman, C.L., Jones, G.W., Gallagher, J., Mallinson, P.J., 1981. The population structure,
453 density and prevalence of tuberculosis (*Mycobacterium bovis*) in badgers (*Meles meles*)
454 from four areas in south-west England. *J. Appl. Ecol.* 18, 795-804.
- 455 Corner, L.A.L., Buddle, B.M., Pfeiffer, D.U., Morris, R.S., 2001. Aerosol vaccination of
456 the brushtail possum (*Trichosurus vulpecula*) with bacille Calmette-Guérin: the
457 duration of protection. *Vet. Micro.* 81, 181-191.
- 458 Corner, L.A.L., Norton, S., Buddle, B.M., Morris, R.S., 2002. The efficacy of bacille
459 Calmette-Guérin vaccine in wild brushtail possums (*Trichosurus vulpecula*). *Res.*
460 *Vet. Sci.* 73, 145-152.
- 461 Corner, L.A.L., Costello, E., Lesellier, S., O'Meara, D., Sleeman, D.P., Gormley, E., 2007.
462 Experimental tuberculosis in the European badger after endobronchial inoculation of
463 *Mycobacterium bovis*: I. Pathology and bacteriology. *Res. Vet. Sci.* 83, 53-62.
- 464 Corner, L.A., Costello, E., Lesellier, S., O'Meara, D., Gormley, E., 2008. Vaccination of
465 European badgers (*Meles meles*) with BCG by the subcutaneous and mucosal routes
466 induces protective immunity against endobronchial challenge with *Mycobacterium bovis*.
467 *Tuberculosis (Edinb)* 88, 601-609.
- 468 De Jong, M.C.M., van der Poel, W.H.M., Kramps, J.A., Brand, A. and van Oirschot, J.T., 1996.
469 Quantitative investigation of population persistence and recurrent outbreaks of bovine
470 respiratory syncytial virus on dairy farms. *Am. J. Vet. Res.* 57, 628-633.

- 471 Diekmann, O., Heesterbeek, J.A.P., 2000. Mathematical epidemiology of infectious diseases:
472 model building, analysis and interpretation. Wiley series in mathematical and
473 computational biology. Chichester, UK: Wiley.
- 474 Eves, J.A., 1999. Impact of badger removal on bovine tuberculosis in east County Offaly. Irish
475 Vet. J. 52, 199-203.
- 476 Fagan, J., 1993. Tuberculosis in badgers in Ireland: Pathology. In: The badger. Proceedings of a
477 seminar held on 6-7 March 1991 pp. 117-122.
- 478 Gallagher, J., Monies, R., Gavier-Widen, M., Rule, B., 1998. Role of infected, non-diseased
479 badgers in the pathogenesis of tuberculosis in the badger. Vet. Rec. 142, 710-714.
- 480 Griffin, J.M., Dolan, L.A., 1995. The role of cattle-to-cattle transmission of *Mycobacterium bovis*
481 in the epidemiology of tuberculosis in cattle in the Republic of Ireland: a review. Irish
482 Vet. J. 48, 228-234.
- 483 Griffin, J.M., Williams, D.H., Kelly, G.E., Clegg, T.A., O'Boyle, I., Collins, J.D., More, S.J.,
484 2005. The impact of badger removal on the control of tuberculosis in cattle herds in
485 Ireland. Prev. Vet. Med. 67, 237-266.
- 486 Halloran, M.E., Struchiner, C.J., Longini, I.M., Jr., 1997. Study Designs for Evaluating Different
487 Efficacy and Effectiveness Aspects of Vaccines. Am. J. Epidemiol. 146, 789-803.
- 488 Halloran, M.E., Longini, I.M., Jr., Struchiner, C.J., 1999. Design and Interpretation of Vaccine
489 Field Studies. Epidemiol. Rev. 21, 73-88.
- 490 Jenkins, H.E., Woodroffe, R., Donnelly, C.A., 2008. The effects of annual widespread badger
491 culls on cattle tuberculosis following the cessation of culling. Int. J. Infect. Dis. 12,
492 457-465.
- 493 Krebs, J.R., 1997. Bovine Tuberculosis in Cattle and Badgers. Ministry of Agriculture, Fisheries
494 and Food, London. UK, 191.
- 495 Lesellier, S., Corner, L., Costello, E., Lyashchenko, K., Greenwald, R., Esfandiari, J., Singh, M.,
496 Hewinson, R.G., Chambers, M., Gormley, E., 2009. Immunological responses and

- 497 protective immunity in BCG vaccinated badgers following endobronchial infection with
498 *Mycobacterium bovis*. *Vaccine* 27, 402-409.
- 499 Longini, I.M., Jr., Sagatelian, K., Rida, W.N., Halloran, M.E., 1998. Optimal vaccine trial design
500 when estimating vaccine efficacy for susceptibility and infectiousness from multiple
501 populations. *Stat. Med.* 17, 1121-1136.
- 502 More, S.J., Good, M., 2006. The tuberculosis eradication programme in Ireland: a review of
503 scientific and policy advances since 1988. *Vet. Microbiol.* 112, 239-251.
- 504 Murphy, D., Gormley, E., Costello, E., O'Meara, D., Corner, L.A.L., 2010. The prevalence and
505 distribution of *Mycobacterium bovis* infection in European badgers (*Meles meles*) as
506 determined by enhanced post mortem examination and bacteriological culture. *Res. Vet.*
507 *Sci* 88, 1-5.
- 508 Noonan, N.L., Sheane, W.D., Harper, L.R., Ryan, P.J., 1975. Wildlife as a possible reservoir of
509 bovine TB. *Irish Vet. J.* 29, 1.
- 510 Olea-Popelka, F.J., Fitzgerald, P., White, P., McGrath, G., Collins, J.D., O'Keeffe, J., Kelton,
511 D.F., Berke, O., More, S., Martin, S.W., 2009. Targeted badger removal and the
512 subsequent risk of bovine tuberculosis in cattle herds in county Laois, Ireland. *Prev. Med.*
513 *Vet.* 88, 178-184.
- 514 Pope, L.C., Butlin, R.K., Wilson, G.J., Woodroffe, R., Erven, K., Conyers, C.M., Chris, M.,
515 Franklin, T., Delahay, R.J., Cheeseman, C.L., Chris, L., Burke, T., 2007. Genetic
516 evidence that culling increases badger movement: implications for the spread of bovine
517 tuberculosis. *Mol. Ecol.* 16, 4919-4929.
- 518 Tompkins, D.M., Ramsey, D.S.L., Cross, M.L., Aldwell, F.E., de Lisle, G.W., Buddle, B.M.,
519 2009. Oral vaccination reduces the incidence of tuberculosis in free-living brushtail
520 possums. *Proc. R. Soc. B* 276, 2987-2995.
- 521 Woodroffe, R., Gilks, P., Johnston, W.T., Le Fevre, A.M., Cox, D.R., Donnelly, C.A., Bourne,
522 F.J., Cheeseman, C.L., Gettinby, G., MCInerney, J.P., Morrison, W.I., 2007. Effects of

523 culling on badger abundance: implications for tuberculosis control. *Journal of Zoology*,
524 28-37.

525 Watchorn, R.C., 1965. Bovine tuberculosis eradication scheme 1954-1965. Department of
526 Agriculture and Fisheries. Dublin.

Accepted Manuscript

527

528

529

530

531

532

533

534

535

536

537

538

539

540

541

542

543

544

545

546

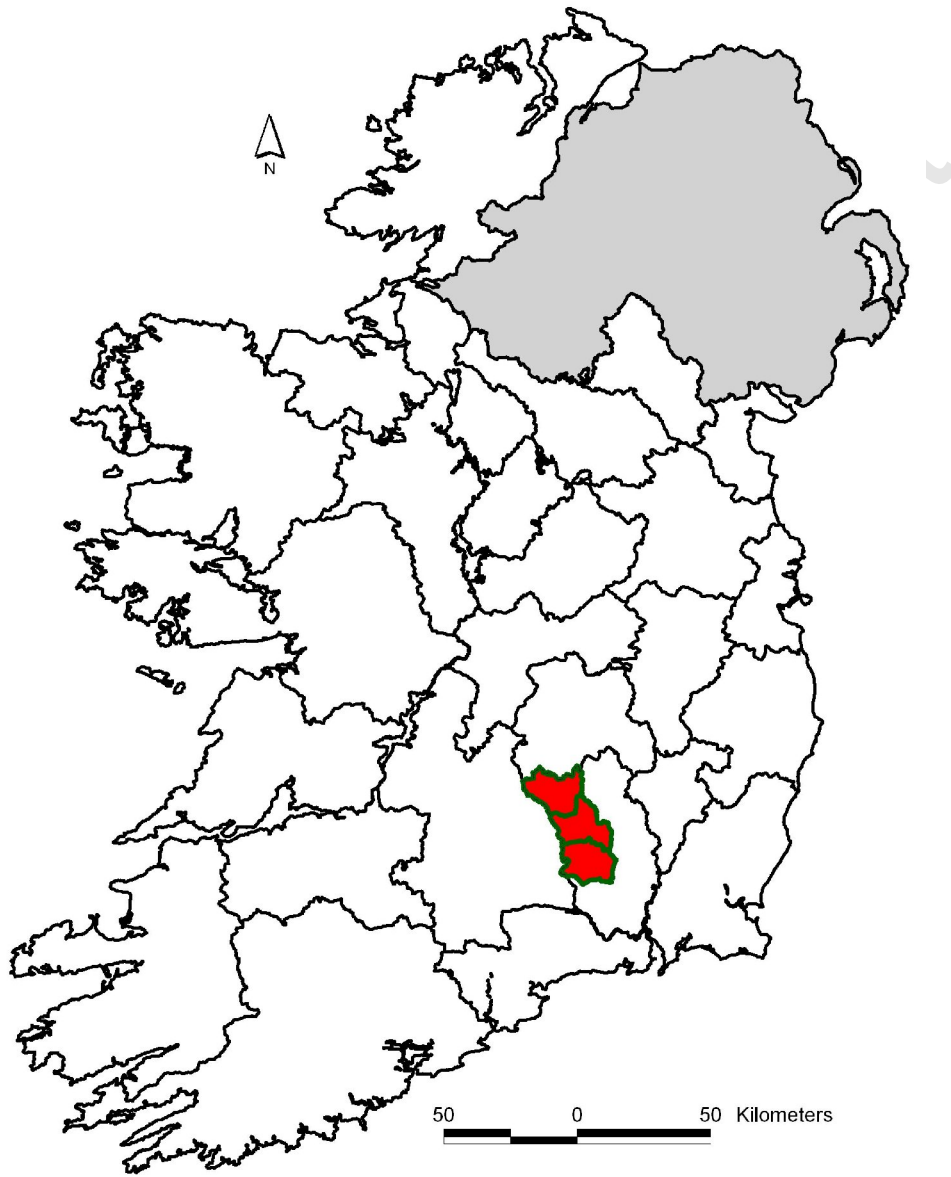


Figure 1. Study site selected for the vaccine trial is highlighted in red

547

548

549

550

551

552

553

554

555

556

557

558

559

560

561

562

563

564

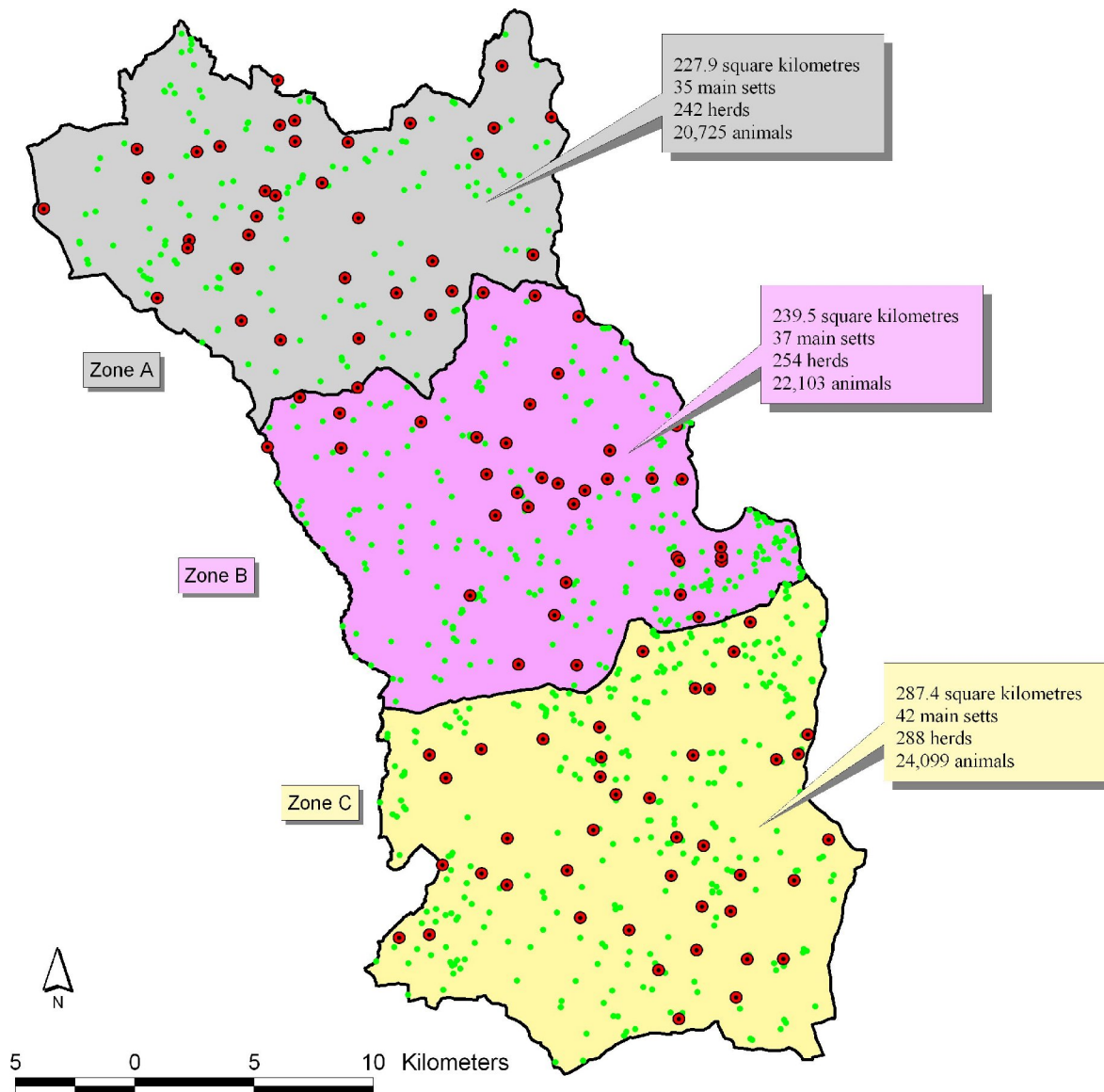
565

566

567

568 **Figure 2.** Map showing study area divided into three zones A, B and C (grey, pink and yellow

569 respectively) where vaccination coverage will be of 100, 50 and 0%. The gradient of coverage



570 (100% to 0% from north to south, or vice-versa) will be allocated randomly at the start of the
571 trial. Main badger setts are represented with red dots and green dots represent other sett types
572

Accepted Manuscript