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

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1 Abstract

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

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

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

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

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

2 Introduction

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

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

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

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

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

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

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

3 Theoretical basis: a review

3.1 Initial considerations in designing a vaccine trial

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

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

3.2 Parameters of effect

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

- transmission from a vaccinated to a vaccinated badger (β_{VV});
- transmission from a vaccinated to an unvaccinated badger (β_{VU});
- transmission from an unvaccinated to a vaccinated (β_{UV}), and
- 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

has to be cautiously interpreted as vaccine protection could be dependent on the infection dose. It is not known what the infectious dose is in natural infections. In field vaccine trials in possum in New Zealand, using conjunctival and intranasal vaccination (Corner et al., 2002) and more recently vaccination by oral delivery (Tompkins et al., 2009), significant protection against natural exposure was seen in the vaccinated group compared to the control groups. Protection was much higher than predicted from previous studies where possums had been experimentally challenged (Corner et al., 2001; Buddle et al., 2006).

Estimation of $R(p)$, VE_S and VE_I will give us a more detailed understanding of the ways BCG vaccine works in a wild badger population.

4 Epidemiological contribution to the design of the vaccine trial

4.1 Study site

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

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

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

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

4.2 Trial design

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

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

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

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

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

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

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

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

5 Analysis of the vaccine trial data

5.1 General description

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

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

- Time interval (Δt) between the two captures

- Disease status of the badger at the beginning and at the end of the time interval
- Vaccination status of the badger
- Zone where the animal was observed at the beginning and end of the time interval (A, B or C)
- Average prevalence of infection in the zone where the badger was caught during Δt (PrevA, PrevB, PrevC)
- Average fraction of infected badgers that are vaccinated during Δt in the zone where the badger was caught (Fv_A , Fv_B , Fv_C)

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

5.2 Estimation of the transmission parameters

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

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

Where:

β = Transmission parameter

Δt = Time interval

I= Number of infected individuals*

N= Total number of individuals

I/N= Prevalence of infected badgers*

* For our purpose infected badgers are deemed to be infectious

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

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

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

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

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

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

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

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

If we assume that there is a multiplicative effect on *NIC* as the fraction of infected vaccinated 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 the

359 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

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

363

364 Since we will know the observed infection status of the specific badger (C_V) at the end of the time

365 interval (Δt), we can fit a GLM with Log (PrevA Δt) as an offset and calculate K_0 and K_1 . We can

366 subsequently calculate β_{UV} and β_{VV} as:

367

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

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

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

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

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 not
380 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

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

385

386 We can also calculate Vaccine Efficacy for Infectiousness as:

387

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

389

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

391

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

393

394

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

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

6 Concluding remarks

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

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

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

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

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

7 Conflict of Interest Statement

The authors have not declared any conflict of interest.

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

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