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Quantification of *Mycobacterium bovis* transmission in a badger vaccine field trial

I. Aznar[a,b,c,*], K. Frankena[b], S.J. More[a], J. O'Keeffe[c], G. McGrath[a], M.C.M de Jong[b]

[a] UCD Centre for Veterinary Epidemiology and Risk Analysis, UCD School of Veterinary Medicine, University College Dublin, Belfield, Dublin 4, Ireland
[b] Quantitative Veterinary Epidemiology group, Wageningen Institute of Animal Sciences, Wageningen University & Research, P.O. Box 338, 6700 AH Wageningen, The Netherlands
[c] Department of Agriculture, Food and the Marine, Kildare St., Dublin 2, Ireland

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ABSTRACT

In the UK and Ireland, Bacille Calmette-Guérin (BCG) vaccination of badgers has been suggested as one of a number of strategies to control or even eradicate *Mycobacterium bovis* infection in badgers. In this manuscript, we present the results of a badger field trial conducted in Ireland and discuss how the novel trial design and analytical methods allowed the effects of vaccination on protection against infection and, more importantly, on transmission to be estimated. The trial area was divided into three zones North to South (A, B and C) where vaccination coverages of 0, 50 and 100%, respectively, were applied. Badgers were trapped over a 4 year period. Badgers were assigned to either placebo or vaccine treatment, with treatment allocation occurring randomly in zone B. Blood samples were collected at each capture, and serology was performed in these samples using a chemiluminescent multiplex ELISA system (Enfer test). The analysis aimed to compare new infections occurring in non-infected non-vaccinated badgers to those in non-infected vaccinated ones, while accounting for the zone in which the badger was trapped and the infection pressure to which this individual badger was exposed. In total, 440 records on subsequent trappings of individual non-infected badgers were available for analysis. Over the study period, 55 new infections occurred in non-vaccinated (out of 239 = 23.0%) and 40 in vaccinated (out of 201 = 19.9%) badgers. A Generalized Linear Model (GLM) with a cloglog link function was used for analysis. Statistical analysis showed that susceptibility to natural exposure with *M. bovis* was reduced in vaccinated compared to placebo treated badgers: vaccine efficacy for susceptibility, VE_S, was 59% (95% CI = 6.5%–82%). However, a complete lack of effect from BCG vaccination on the infectivity of vaccinated badgers was observed, i.e. vaccine efficacy for infectiousness (VE_I) was 0%. Further, the basic reproduction ratio as a function of vaccination coverage (p) (i.e. R(p)) was estimated. Given that the prevalence of *M. bovis* infection in badgers in endemic areas in Ireland is approximately 18%, we estimated the reproduction ratio in the unvaccinated population as R(0) = 1.22. Because VE_S was now known, the reproduction ratio for a fully vaccinated population was estimated as R(1) = 0.50. These results imply that with vaccination coverage in badgers exceeding 30%, eradication of *M. bovis* in badgers in Ireland is feasible, provided that the current control measures also remain in place.

1. Introduction

Bovine tuberculosis (bTB, caused by infection with *Mycobacterium bovis*) is a chronic inflammatory disease of bovidae (Bezos et al., 2014). A control/eradication programme for bTB in cattle started in Ireland in 1959 not only to address the economic losses associated with the infection (Caminiti et al., 2016), but also its zoonotic potential (Langer and LoBue, 2014). In the first ten years of the control programme, with a focus on measures to limit cattle to cattle transmission, the incidence of *M. bovis* infection in cattle was reduced from 17% to 0.5% (More and Good, 2006). Subsequently, progress has been slow, despite ongoing application of intense control strategies, which raised concerns about a role for one or more reservoirs of *M. bovis* maintaining transmission. Over the years, this hypothesis has been confirmed, including work highlighting high prevalence of infection in badgers (*Meles meles*) (Corner et al., 2005). Since then substantial research has been conducted to understand transmission of *M. bovis* between cattle and badgers, and of potential strategies capable of reducing this transmission. One such strategy is the use of BCG (Bacille Calmette-Guérin) badger vaccination (More and Good 2006).

* Corresponding author at: Centre for Veterinary Epidemiology and Risk Analysis, UCD School of Veterinary Medicine, University College Dublin, Belfield, Dublin 4, Ireland.
E-mail address: inma.aznar@ucd.ie (I. Aznar).

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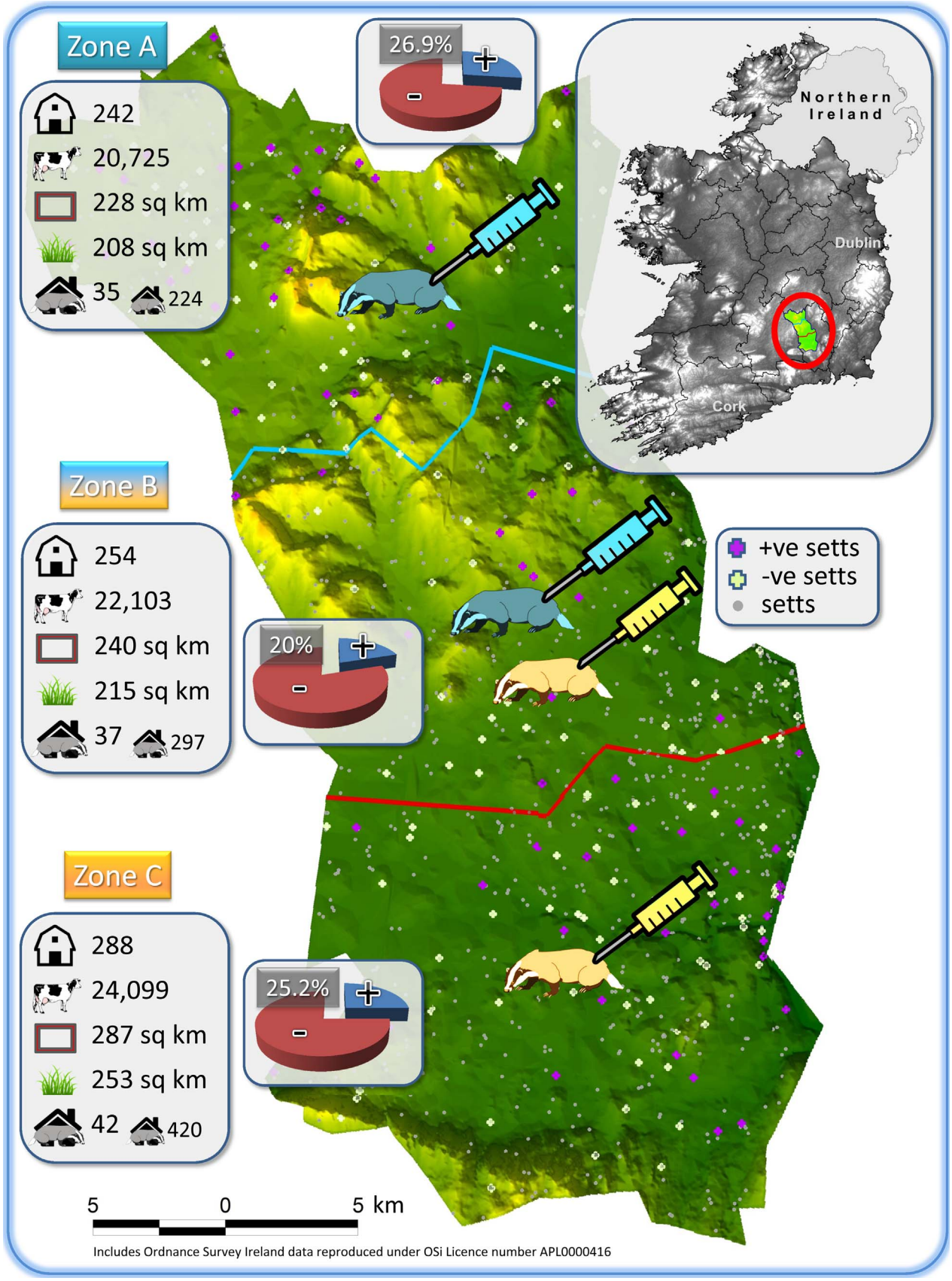

Fig. 1. Topographic map of the Irish badger vaccine field trial showing: number of farms and bovines, zone area and area of farmed grassland (sq km), and number of main and secondary badger setts per zone. From north to south, zones A, B and C indicate vaccine (blue badger) and/or placebo (yellow badger) allocation. Estimated *M. bovis* prevalence in badgers at the end of the first year is shown per zone (pie charts). Badger setts are represented as: all surveyed setts (grey dots), setts with at least one positive badger trapped in the first year (purple cross), setts with at least one negative badger trapped in the first year (light green cross). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Experimental challenge trials with *M. bovis* following BCG vaccination by subcutaneous, mucosal, oral or intramuscular routes (Corner et al., 2008; Lesellier et al., 2009, 2011; Murphy et al., 2014) have demonstrated a reduction in disease progression in captive badgers. It has been proposed that this observed reduction in the number of sites with gross pathology and of general gross pathological severity scores observed in these badgers, could translate to a reduction of badger infectivity, and thus to a reduction in transmission in the field (Chambers et al., 2011). Here, the expected reduction in transmission due to a lower infectivity of badgers equates to what is known as vaccine efficacy for infectivity (VE_I). In the human field, it is not uncommon to find vaccines that, by helping to reduce pathology and clinical symptoms in vaccinated and subsequently infected individuals, achieve a reduction of the infectivity of these individuals and, as a consequence, a reduction in transmission in the general population. Vaccines against smallpox, varicella, rubella, measles, hepatitis B and whooping cough have been recognized as having an important VE_I which contributes to the overall effect of these vaccines on the population (vaccinated and non-vaccinated), this overall effect being referred to as herd immunity (Fine, 1993; Halloran et al., 1999; Stephens, 2008).

In addition, protection of badgers against *M. bovis* infection could also be achieved as a consequence of reduced susceptibility. A reduction in susceptibility against infection would have both a direct and an indirect effect in the general population, i.e. vaccinated individuals are less likely to become infected (direct effect) and therefore, non-infected badgers are less likely to become infected if surrounded by these less susceptible individuals (indirect effect). Although this type of protection was not observed in laboratory trials, a reduction in susceptibility could potentially be attained under natural conditions because the infective dose that badgers are exposed to in the field is likely to be much lower than that used in experimental trials (Corner et al., 2008; Lesellier et al., 2011). This type of protection is referred to as vaccine efficacy for susceptibility (VE_S). VE_S solely refers to the direct effect.

Knowledge of both vaccine efficacies is important as overall transmission depends on both susceptibility and infectivity. However, methods to quantify transmission after vaccination have only been used in the last 20 years (Moerman et al., 1993; Stegeman et al., 1995; De Jong and Kimman, 1994). In 1994, de Jong and Kimman designed an experimental study that allowed quantification of the transmission observed in pigs vaccinated against pseudorabies virus. In subsequent experimental and field transmission studies, the effectiveness of vaccination was evaluated based on estimation of R(p) or the basic reproduction ratio as a function of the proportion of the population that is vaccinated (Moerman et al., 1993; Stegeman et al., 1995). R(p) is a crucial parameter to understand the impact of vaccination on population dynamics of *M. bovis* infection. If BCG vaccination is capable of reducing transmission between badgers, then estimates of the minimum vaccine coverage necessary to achieve eradication in badgers would be essential when designing an eradication programme, based on Diekmann et al. (1990). By examining R(p), the effects of combining vaccination with other control methods in the same or different species (e.g. the strategy of detection-and-removal of infected cattle from cattle herds) can be calculated. This is extremely important in the case of vaccination in badgers, as the ultimate goal is to help in the control or eradication of *M. bovis* infection in cattle.

Aznar et al. (2011) presented a novel design of a badger vaccination trial and developed a methodology to estimate both VE_S and VE_I as well as R(p) based on incidence data (i.e. new *M. bovis* infections). The trial design consisted of three badger populations vaccinated with different vaccination coverages as suggested by Longini et al. (1998), but taking into account that these vaccination coverages are achieved over time rather than instantaneously. Here, we present the results of this badger vaccine/placebo field trial. *M. bovis* transmission among badgers was quantified as well as the effects of vaccination on the susceptibility and infectivity of badgers. Based on these results, the impact of badger vaccination on the *M. bovis* eradication programme in Ireland is reviewed.

2. Material and methods

2.1. Trial

The badger vaccine field trial ran from 2009 until 2013. The trial area of approximately 750 square kilometres was divided into three zones north to south (A, B and C respectively) (see Fig. 1). Using cages and stopped wire restraints, a capture–tag–release regime was established. Traps were fitted and left in the vicinity of every active sett for 10 days, with daily checks carried out by DAFM (Department of Agriculture, Food and the Marine) employees. After the 10 day period, traps were moved to different setts, taking approximately 23 weeks to cover the whole trial area (trappings occurring simultaneously in all three zones). Each 23 week period constituted a “sweep”. A total of 8 sweeps were carried out over the length of the trial (2 sweeps per year). The last two sweeps involving some badger removal to allow for post-mortem evaluations (in the study of Gormley et al. (2017), sweeps 7 and 8 were combined and presented as sweep 7).

At first capture, each badger was tattooed and microchipped, with blood samples being collected at first capture and every subsequent recapture (Gormley et al., 2017). Vaccination with an oral BCG vaccine (Danish strain 1331, at a dose of 1×10^8 cfu of BCG administered in the upper pharyngeal mucosa) suspended on a lipid formulation (Ancelet et al., 2012; Gormley et al., 2017) was applied randomly to 50% of the badgers trapped in zone B and to all badgers trapped in zone C. All badgers in zone A and the remaining 50% of the badgers trapped in zone B received a placebo.

The Enfer chemiluminescent multiplex ELISA system (Whelan et al., 2008, 2010; Aznar et al., 2014) had been previously optimized to be used as the diagnostic test in this trial. The Enfer test optimization was conducted using data obtained from a population of 215 badgers trapped across 16 counties in Ireland (Murphy et al., 2010; Aznar et al., 2014). These badgers had been thoroughly examined and a large number of samples from tuberculous and non-tuberculous lesions were taken for culture (culture was used as the gold standard). Details about these badgers and culture methods are presented in Murphy et al. (2010). A study of factors affecting the statistical power of this design highlighted the importance of achieving close to 100% specificity in the diagnostic test used (Aznar et al., 2013). Therefore, the Enfer test was optimized to maximise sensitivity while retaining specificity at 99.99% in order to avoid loss of power that would arise from a number of false positive results randomly occurring in the mainly negative samples from both vaccinated and unvaccinated animals (Aznar et al., 2014). Test sensitivity did not play a major role in terms of study power, however, there was a need for consistent test performance among samples from all study animals throughout the trial period, including those vaccinated and not vaccinated. Many steps were taken to achieve this, including evaluating and comparing test results from vaccinated and non-vaccinated animals from experimental studies. Differences in terms of time to seroconversion were observed when the Enfer test was applied to vaccinated and non-vaccinated captive badger groups. As a result, a minimum time lag between two subsequent trappings of 215 days for all pairs of trappings was recommended (Aznar et al., 2014).

The trial was carried out under three licences issued by three different bodies: the Department of Health & Children (research licence, B100/3187), the Department of Agriculture, Food & the Marine (clinical trial licence, RL/08/06) and the Animal Research Ethics Committee of University College Dublin (ethics approval, AREC-P-08-26).

2.2. Datasets

Two datasets were collected for analysis. The first consisted of data

collected in the field (using handheld computers) by operators in charge of capturing and treating badgers in the trial area. This dataset contained information on 2189 badger trappings (from the 1st of September 2009 to the 12th of July 2013). Data recorded on the handheld computers prior to the start of the trial and during the operator's training period were discarded (133 trapping records). Information recorded at each of the trappings were: badger identification (ID) data (badger ID, microchip and tattoo numbers), badger's sett ID, date of examination, presence of ectoparasites (ticks, fleas, lice) and injuries, demographic data (age, sex, weight), type of diagnostic samples taken (faecal swabs, blood samples, pharyngeal swabs, DNA samples, others), vaccination data (date of vaccination, vaccine code), operator name, comments, trial zone (A, B or C), and sweep number (1–8).

The second dataset, consisting of 1800 records, contained diagnostic test information of blood samples taken each time a badger was trapped. Blood samples were tested using the Enfer multiple antigen ELISA system for detection of *M. bovis* antibodies (Enfer Scientific, Co. Kildare, Ireland). Antibody responses were expressed as relative light units (RLU) to a panel of 8 antigens: MPB83, MPB70, Rv3616c fragment and full protein, ESAT-6 and CFP10, as well as purified protein derivative from *M. bovis* (PPDb) and a peptide of MPB70. The optimization process is described in detail by Aznar et al. (2014). Blood samples were analysed twice with the Enfer test: first after the end of each sweep, and a second time after the vaccine trial had ended. When both sets of results were compared, low repeatability for two antigens (MPB70 and Rv3616c fragment) was observed. These two antigens were removed prior to the final test optimization. The optimization was carried out using the second set of test results and after removing the two mentioned antigens. For that, a stepwise logistic regression with analytical weights (to optimize specificity versus sensitivity) on the converted RLU obtained for the six remaining antigens was carried out (Aznar et al., 2014). By assessing the ROC curve for the model results, a cut off value equal to −1.95 was selected to achieve 99.99% (exact confidence interval: 97.34–100%) specificity and 25.33% (exact confidence interval: 20.80–42.24%) sensitivity (exact intervals instead of confidence intervals were calculated as specificity was very close to 100%). Blood samples were classified as positive or negative based on this cut off value.

2.3. Data collation

The two datasets, containing capture and serology data were merged (1759 trapping records). Data were collated to be analysed as a Bernoulli experiment. For that purpose, the full dataset was organized so that each entry contained information regarding two subsequent trappings of a single badger (vaccinated or non-vaccinated) that tested negative at the initial trapping. The first entry for an individual badger was recorded the second time that a specific badger was trapped. A badger that tested negative at its second trapping could then initiate a new record in our dataset if trapped for a third time, and so forth. Each entry line contained information on: infection status of the badger at the initial and current trapping, current and previous examination date, sweep number and zone where the badger was trapped each time, whether the badger had been allocated to vaccine or placebo, and date of treatment. Once a badger was allocated to either vaccine or placebo, it remained as such for the rest of the study. Prior to the analysis, three variables were calculated from the data recorded in the handheld computers including: *delta t* (Δt) (i.e. time in days between two subsequent trappings of an individual badger), and prevalence (*Prev*) and fraction of infected vaccinated badgers (*Fi*; the fraction of the total number of infected badgers that became infected after vaccination) at the beginning of Δt in the zone where the badger was trapped. A badger allocated to the vaccine treatment was considered vaccinated the day after receiving the vaccine. Therefore, as we knew whether the badger had been allocated to the vaccine or placebo treatment, a new variable *Vaccine status* (*Vs*) was created that coded 0 for badgers allocated to the placebo treatment (also for badgers allocated to the vaccine treatment on the first date of treatment) and 1 for vaccinated badgers trapped at least one day after they received the first vaccination.

2.4. Statistical analysis

The data collation, as well as the descriptive and statistical analyses, were carried out using Stata® (version 14; Stata Corp., College Station, TX, USA). As part of the descriptive analysis, crude transmission rate parameters (beta transmission parameters) were calculated as the number of new cases divided by number of susceptibles and prevalence in each sweep, for the three zones. In order to help in visualizing patterns, a non-parametric regression of the beta transmission parameters (lowess smoothing) was conducted.

The purpose of the statistical analysis was to compare new infections occurring in vaccinated non-infected badgers to those occurring in non-vaccinated non-infected ones while taking account of both the infection pressure these badgers were exposed to and the trial zone (A, B or C) badgers had been trapped in (Aznar et al., 2011). Data on 440 pairs of trappings (subsequent trappings of individual badgers) were used in the statistical analysis. Only badgers that tested negative at the initial trapping were included. Badgers were coded either 1 or 0, respectively, depending on whether or not they tested positive at the subsequent trapping.

Assuming "separable mixing", whereby transmission depends only on the infectivity of the donor and the susceptibility of the receptor (Diekmann et al., 1990), the expected infection status of any uninfected re-trapped badger (vaccinated or non-vaccinated) was modelled in the total population using a generalized linear model (GLM). With this model we aimed to explain new infections from three explanatory variables: a) the vaccination status of the badger, b) the fraction of infected vaccinated badgers, and c) the zone where the badger was trapped. Details of the statistical model are elaborated below. If vaccination is effective, then we would expect infectivity to vary both between the three zones and also over time due to differences in the fraction of infected badgers that were vaccinated. It is important to note that the percentage of vaccinated badgers increased over the duration of the trial in zones B and C (Fig. 2).

The expected number of cases per unit of time $E(C)$ can be formulated as $E(C) = S\cdot(1 - e^{-\beta\cdot\Delta t\cdot Prev_t})$ where S is the number of susceptible badgers and $\left(1 - e^{-\beta * I * \frac{\Delta t}{N}}\right)$ is the probability that any of the susceptible badgers becomes infected (supplementary material, Section 1). Then the complementary log–log (cloglog) link function results in an estimate for log (β) taking ln(*Prev**Δt) as offset (Aznar et al., 2011). This model was run separately for vaccinated and non-vaccinated badgers, allowing separation of the effects of vaccination in susceptibility and in infectivity as explained in derivations presented in Section 1 of the supplementary material. By separating these two effects, estimations of VE_S and VE_I are possible. The model used was:

$$cloglog\ E(C) = \beta_0 + \beta_{1,B}Z_B + \beta_{1,C}Z_C + \beta_2 Vs + \beta_3\ Fi + of\ fset$$

where *Z* codes for zone (binary dummy variable 0/1 for each of the zones, zone A being the reference), vs is the vaccination status of the recipient badger, *Fi* is the fraction of vaccinated badgers among the infected badgers at the beginning of the time interval in that same zone, and β_0, $\beta_{1,B}$, $\beta_{1,C}$, β_2, β_3, are the regression coefficients as estimated by our model. For modelling purposes, once a badger tested positive to the serological test, it was considered positive for the rest of the study and therefore subsequent trappings of this badger were not included in the analysis. As the number of predictors in the maximum model was small, all possible combinations of predictors were examined (including interaction terms). The final model was selected based on the lowest value for the Akaike Information Criterion (AIC).

From this model, four transmission parameters: β_{vv}, β_{vu}, β_{uv} and β_{uu} were estimated. The first sub-index in these transmission parameters

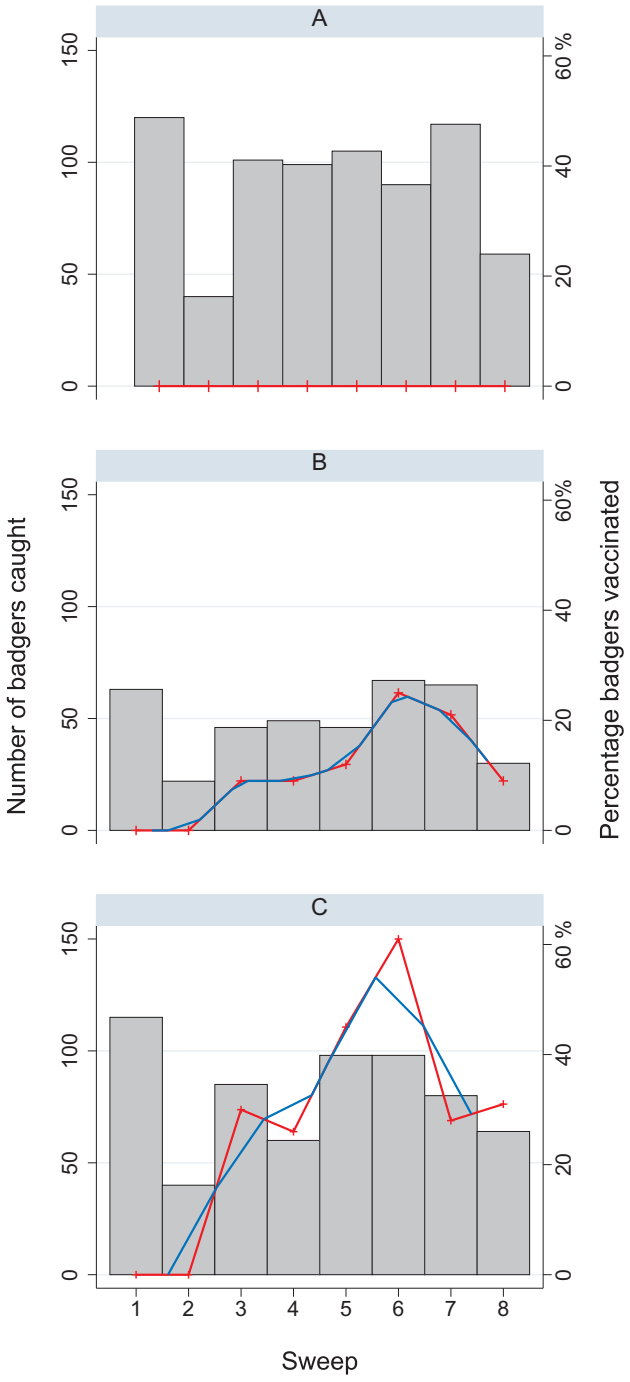

Fig. 2. Total number of badgers caught at each sweep (left vertical axis) and percentage of captured badgers that were vaccinated (right vertical axis), including polynomial (n = 4) smoothing of this percentage per sweep and zone (right vertical axis).

indicates the vaccination status of the badger transmitting *M. bovis* (whether it is from a vaccinated (v) or non-vaccinated badger (u)), while the second sub-index refers to the vaccination status of the recipient badger. The two vaccine efficacies and R(p) can then be calculated from these four transmission parameters (see Section 1 of the supplementary material). Using the regression coefficients from our model, the transmission rate parameters, ignoring zone effects, can then be estimated as:

$$\beta_{uv} = e^{\beta_0} + \beta_2,\ \beta_{vv} = e^{\beta_0} + \beta_2 + \beta_3,\ \beta_{uu} = e^{\beta_0} \text{ and } \beta_{vu} = e^{\beta_0} + \beta_3$$

Vaccine efficacies were calculated as:

$$VE_s = 1 - \frac{\beta_{uv}}{\beta_{uu}} = 1 - \frac{\beta_{vv}}{\beta_{vu}} = 1 - e^{\beta_2} \text{ and } VE_I = 1 - \frac{\beta_{vu}}{\beta_{uu}} = 1 - \frac{\beta_{vv}}{\beta_{uv}} = 1 - e^{\beta_3},$$

noting that coefficient β_2 calculated for the variable (*Vs*) contributes to the estimation of VE_s, and the coefficient β_3 calculated for the variable (*Fi*) contributes to the estimation of VE_I, thus being able to estimate both vaccine efficacies. The reproduction ratio as a function of the proportion (p) of badgers vaccinated R(p) was determined as:

$$R(p) = (1 - p) \cdot R(0) + p \cdot R(1)$$

Where $R(0) = \frac{1}{1 - \text{prevalence}}$ and $R(1) = (1 - VE_S) \cdot (1 - VE_I) \cdot R(0)$

3. Results

3.1. Vaccine field trial descriptive analysis

Overall, 1093 badgers were trapped over the 8 sweeps, with 435 badgers trapped in zone A, and 243 and 415 in zones B and C, respectively. In total, 673 badgers were trapped once, 253 twice, 111 three times, 38 four times, 13 five times and 5 six times. An initial concern over the vaccine trial design was the fact that no major physical boundaries existed between the three zones. A large number of badger movements across the three zones could have hampered the vaccination gradient between the zones and therefore reduced the power of the analysis. Such a large movement was not expected, nonetheless we can confirm that it did not occur as only in 2% (22) of the subsequent trapping events had badgers originally trapped in one zone been trapped in a different zone at a later stage.

The prevalence of *M. bovis* infection, estimated as the overall percentage of positive trappings to the Enfer test at each sweep, ranged between 12.5% and 37.8% (see Table S1 in Section 2 of the supplementary material). At the beginning of the trial, the zone prevalence (the percentage of positive trappings in each zone in sweep 1) was higher, but not statistically different, in zone A (31.7%) compared to zones B (19.0%) and C (23.5%) (p-value = 0.14). During the first year of the trial (that is, considering sweeps 1 and 2 together to avoid the effect of seasonality on trapping efforts), the prevalence was also highest in zone A (26.9%) compared to zones B and C (20% and 25.2%, respectively) but again, these differences were not statistically significant (p-value = 0.49). Due to a procedural error, blood results for 70 samples taken from badgers during sweep 2 were not available (see Section 4 of the supplementary material). The incidence of *M. bovis* infection per sweep, defined as the number of newly infected badgers (captured badgers that tested positive for the first time in sweep n) divided by the number of susceptible badgers (badgers trapped in sweep n that had never tested positive or tested positive for the first time in that sweep), varied over time and across zones, with the lowest incidence being in sweep 5 in zone C (see Fig. S1 in Section 2 of the supplementary material). In zones B and C, the proportion of BCG vaccinated badgers increased from sweep 3–6, then decreased in sweeps 7 and 8 (as the last sweeps involved badger removal) (Gormley et al., 2017). At sweep 6, the proportion of vaccinated badgers in zones B and C were 37.3% and 62.2% respectively (Fig. 2).

Crude transmission rate parameters in each sweep, for the three zones, and a lowess smoothing of the transmission parameters are presented in Fig. 3. During the trial, there was a non-significant decrease in these crude transmission rate parameters in zones B and C. The possible change over time in crude transmission rate was less clear for zone A. However, the overall initial transmission (at sweep 3) in this zone (i.e. even before vaccination could have had an impact) was already lower compared to the other two zones (Fig. 3).

3.2. Statistical analysis

The dataset consisted of 440 records (239 originated from non-

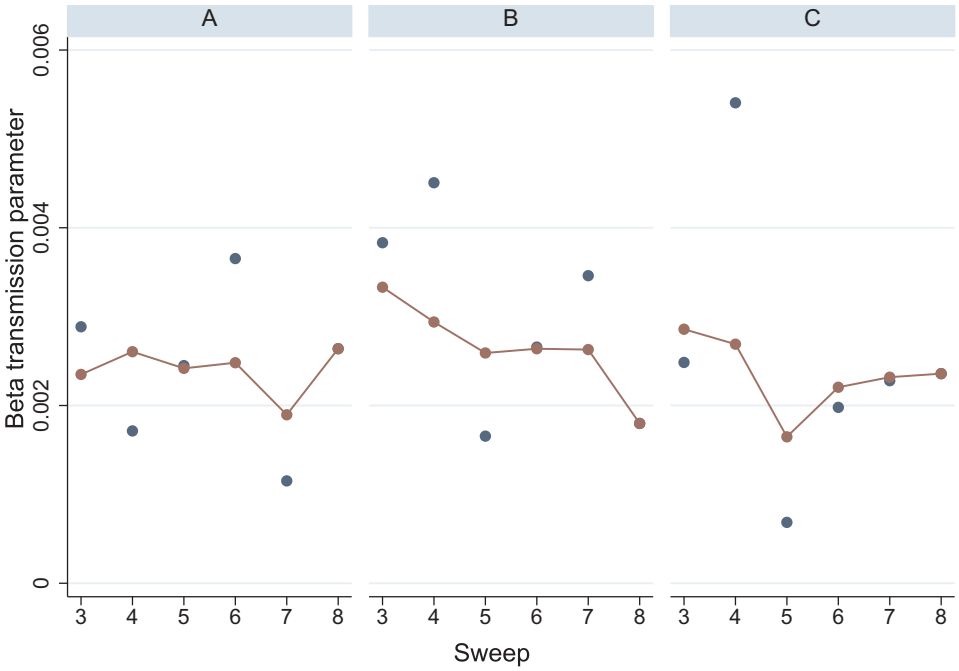

Fig. 3. Crude transmission rate parameters (beta, in blue) and lowess smoothing (in red) per sweep estimated separately for the three trial zones. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Table 1
Results of the final generalized linear model including the estimated regression coefficient, *p*-value and 95% confidence interval for all explanatory variables (fraction of infected vaccinated badgers, vaccination status and zone) and constant in a model fitted in data from all three zones of the Irish badger vaccine field trial. Only Vs, the vaccination status of the recipient, is significant, with the other variables retained to control for confounding.

Variable	Coef	p-Value	(95% CI)
Constant	−6.07	< 0.001	−6.38 to −5.77
Zone			
A	Reference		
B	0.55	0.083	−0.07 to 1.17
C	0.63	0.193	-0.32 to 1.58
Vs	−0.90	0.034	−1.73 to −0.07
Fi	1.37	0.119	−0.35 to 3.10

vaccinated badgers and 201 from vaccinated badgers). A total of 55 (23.0%) and 40 (19.9%) new infections occurred in non-vaccinated and vaccinated badgers, respectively. Vaccination status of the badger receiving the vaccine was the only statistically significant explanatory variable in the model. Nonetheless, all variables (except the interaction terms) were kept in the final model as that was the model with the lowest AIC (490.2). Using the coefficient obtained for recipient vaccination status, we calculated vaccine efficacy for susceptibility, VE_S, as 59% (95% CI = 6.5–82%); that is, a 59% reduction in susceptibility of vaccinated compared to unvaccinated badgers was achieved (see Table 1), but no significant effect of vaccination on infectivity was observed in this trial (hence VE_I = 0%).

In addition to the main model, two more statistical analyses were conducted. As Zone B resembles the classic 50:50 vaccine-placebo trial design (but with a change in vaccination coverage over time and the availability of longitudinal data on infection), estimation of the direct effect of vaccination on susceptibility was possible in this zone only. The model showed a similar outcome for VE_S (54%, 95% CI = 0.0–79.9%) (see Table S2 in Section 3 of the supplementary material). A lower initial crude beta transmission parameter was observed in zone A compared to zones B and C (Fig. 3), for reasons that are not clear. Due to this lower initial transmission parameter observed in zone A, and as our design only required a minimum of two populations vaccinated at different vaccination coverages, the model was run again using data from zones B and C only. Similar results were obtained in terms of both the effect of vaccination on susceptibility and infectivity, with VE_S = 60% (95% CI = 8.8-83.0%) and no significant effect of vaccination on infectivity (VE_I = 0%) (see Table S3 in section 3 of the supplementary material).

We finalized our analyses by estimating R(p) for a range of vaccine coverages, as it is the impact of the combination of both vaccine efficacies that determines the feasibility of using vaccination as a strategy to achieve *M. bovis* eradication in badgers. The average *M. bovis* prevalence in badgers in Ireland declined between 2007–2013, with an average prevalence from May 2007 to May 2011 equal to 17.7%, and from June 2011 to April 2013 equal to 9.9% (Byrne et al., 2015). For any population where an infection is at the endemic steady state, the fraction of susceptible individuals equals 1/R. Thus, for a badger prevalence equal to 18%, we can calculate $R(0) = \frac{1}{1-\text{prevalence}} = 1.22$, and for a prevalence equal to 10%, R(0) = 1.11. As VE_S = 59% and using the higher prevalence estimate (18%), the reproduction ratio for a fully vaccinated population can be calculated as $R(1) = (1 - VE_S)^*R(0) = 0.50$. These results indicate that adding vaccination to the current control strategies in Ireland, eradication of *M. bovis* infection in badgers can be achieved with any vaccination coverage above 30% (Fig. 4).

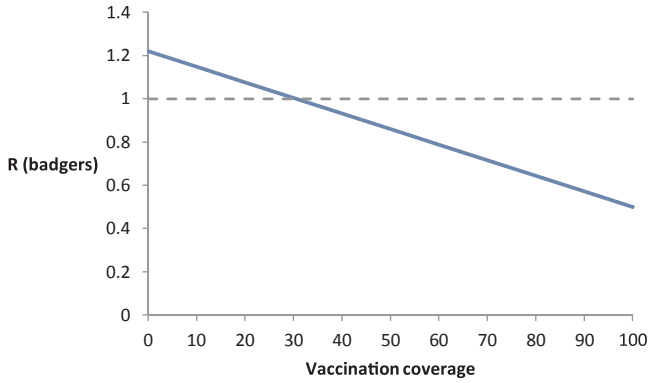

Fig. 4. Basic reproduction ratio for badger to badger transmission as function of vaccination coverage, given R(0) = 1.22 and a VE_S = 59%.

4. Discussion

In this manuscript, the effect of BCG vaccination on *M. bovis* transmission between badgers in the field has been quantified for the first time. Here, separate estimates on the effects of vaccination on both protection against infection and on the infectivity of badgers that become infected subsequent to vaccination are presented. The vaccine efficacy estimates presented in this paper contribute to a better understanding of the biological processes underpinning the protection against transmission achieved by BCG vaccination in the field. While no direct protection against infection following vaccination was reported in experimental trials (where vaccinated badgers were challenged with different doses and different strains of *M. bovis*) (Corner et al., 2008, 2010), we observed a 59% protection against infection of vaccinated badgers in the field. The difference between our findings and those obtained in laboratory trials is not surprising, as the route of infection, infection dose, number of infection events to achieve this dose, etc occurring in the wild are unknown. Nonetheless, we cannot confirm whether or not the observed protection against infection is due to a lower infection dose in the wild compared to experimental trials (Corner et al., 2008; Lesellier et al., 2011). A reduction in the total infectivity of vaccinated and subsequently infected badgers in the field had been anticipated based on the reduction in disease progression observed in vaccinated compared to non-vaccinated badgers in experimental studies (Chambers et al., 2011). However, no reduction of infectivity was found in our study. The lack of effect of BCG vaccination on infectivity in the general badger population is thus at odds with the hypothesis that vaccination, by reducing disease progression, reduces the infectivity of vaccinated and subsequently infected badgers. From this study, we cannot determine whether a similar reduction in disease progression to that observed in experimental studies was found in the field as no post-mortem data were available. Nevertheless, if that reduction in disease progression does exist, we did not find a concurrent reduction in infectivity. The lack of effect of vaccination on infectivity has implications in terms of the effectiveness of BCG badger vaccination in Ireland (or how much reduction of transmission is achieved by vaccination). The effectiveness of a vaccination programme is the result of both the effect of vaccination on susceptibility and infectivity. Here, as there is no added reduction in transmission due to a reduction in infectivity (one type of indirect effect of vaccination), the total reduction in transmission or effectiveness achieved by vaccination is equal to VE_S.

Once the effectiveness of BCG vaccination was calculated, in order to assess its impact, it was necessary to estimate the ongoing badger to badger transmission. The reproduction ratio for badger to badger transmission under the current control options in Ireland was calculated as 1.22 assuming a badger prevalence of 18%. Based on surveillance data collected from badgers culled as part of an interim badger culling regime in Ireland during 2007–2013, an average national prevalence of 14.1% was estimated (Byrne et al., 2015). However, this includes two partial prevalence estimates (17.7% for May 2007 to May 2011, and 9.9% for June 2011 to April 2013), noting that differing methods were used during these periods to differentiate *M. bovis* from non-tuberculous mycobacteria (biochemical tests to May 2011, and PCR techniques subsequently). In this paper, we used 18% as a conservative prevalence estimate. The formulae used for calculating $R(0)$ is a basic formulae used to assess transmission in badgers assuming that there is no transmission between cattle and badgers. Although this is likely not the case, we can use this number as an approximation, and conclude that if we were to vaccinate all badgers in Ireland, we would be able to reduce transmission by 59%, with the resulting $R(1) = 0.5$, which is substantially below 1 indicating that eradication in badgers would be feasible. Further and by estimating $R(p)$ or the reproduction ratio for a range of vaccine coverages (p), we were able to assess what was the minimum vaccination coverage necessary to eradicate. The most relevant finding in this manuscript was that in Ireland, vaccination of badgers with a vaccination coverage equal to or higher than 30% is sufficient to eradicate *M. bovis* infection in badgers, as long as current control strategies also remain in place in both cattle and badgers. The outcomes of this study will have major implications for the control of *M. bovis* infection in Ireland, not only in badgers but also in cattle. It is important to note that if any or some of the control strategies currently in place have an effect on badger to badger transmission, then modifications to any strategy would have repercussions on the effectiveness of the badger vaccination programme (as the reproduction ratio for badger to badger transmission would change also). For similar reasons, it is not possible to predict the effectiveness of BCG vaccination in badgers in a different country, with different transmission characteristics between badgers.

In this study the infection status of individual badgers was determined by whether or not these badgers tested positive to the Enfer test. Table S1 shows the prevalence of infection as measured by this test. Prevalence values varied between sweeps and zones with prevalence in sweep 2 in zone 1 being much lower (12.5%) than that observed in the same zone in sweep 1 (31.7%). The second lowest prevalence observed in the whole study was in zone C in sweep 5 (16.3%) with the rest being between 20.0–37.8%. We are not aware of any specific reasons why these prevalence values changed and we assume that these differences are due to randomness. Tuberculosis is a chronic disease with latent and reactivation periods and with serology varying through the different disease stages. If the badger population in the trial differed in terms of disease profile from the 215 badgers in which the test was optimized (i.e. a larger proportion of badgers in a chronic phase in the field trial), the sensitivity of the Enfer test in the trial could be higher or lower than the 25.3% achieved during test optimization. Indeed, prevalence estimates very much depend on the representativeness of the gold standard panel for the population tested as there is not yet a gold standard test for *M. bovis* infection in badgers. In a previous study where factors affecting study power were explored, it was shown how high test specificity was paramount (Aznar et al., 2013). Test sensitivity did not play an important role in our ability to detect an effect if BCG vaccination really worked. The fact that we found an effect (VE_S = 59%) suggests that both sensitivity and specificity were sufficiently large and did not affect the study power. The low sensitivity of the test used will also have an effect on incidence and prevalence values and therefore on the beta transmission parameter. We note, however, that the aim of this paper was not to provide true values for these parameters, but rather to use them to estimate VE(s) by comparing them in the vaccinated and non-vaccinated groups.

Badger capture data from this vaccine trial has been previously analysed (Gormley et al., 2017). Two vaccine efficacies were reported from this earlier analysis, one for badgers enrolled during sweeps 1 and 2 (VE = 36%) and other for badgers enrolled during sweeps 3–6 (VE = 84%). In that study, the direct effect of vaccination was estimated by comparing hazard rates of badgers trapped in zone A (0% vaccination coverage) to that of badgers trapped in zone C (100% vaccination coverage). In addition to the different serological tests used in both studies (incidence in badgers was measured with the BrockTB Stat-Pak lateral flow serology test, (Chambers et al., 2008)), the methodology in which badgers were enrolled for the analysis and the statistical methods used to compute VE estimates were also different. Data from zone B were not used in the prior analysis, despite badgers from this zone being the ideal population for measuring the direct effect (as vaccinated and non-vaccinated badgers would have been exposed to the same infection pressure).

In a badger vaccine field trial carried out in the UK (Carter et al., 2012), badger setts (rather than individual badgers) were allocated to either vaccine or placebo. From that field study, estimates of the direct effect of BCG vaccination on susceptibility in badgers have been reported with two estimates depending on the diagnostic tests used: VE_S = 54% (95% CI = 12-74%) for the more sensitive test (described as "triple $test_V$") and VE_S = 76% (95% CI = 48-89% for the less

sensitive test ("dual test"). Nonetheless due to the study design of choice, separation of the effects of vaccination in susceptibility and infectivity was not possible in either Gormley et al. (2017) or Carter et al. (2012), leading to two biases in the estimate of VE_S. Firstly, the indirect effect of BCG vaccination is included in the estimate of VE_S (although this estimate should only reflect the direct effect of vaccination), and secondly, if the infectivity of vaccinated and non-vaccinated badgers differs, then this difference in infectivity has to be taken into account also when estimating VE_S. In hindsight, and based on our results, we now know that such a difference does not exist ($VE_I = 0$ as the coefficient for Fi was not statistically significant). Nonetheless, it is important that this issue is highlighted so it can be considered in the design of future vaccine field trials.

A reduction in *M. bovis* incidence in cubs from vaccinated setts compared to those from non-vaccinated setts was also observed in Carter et al. (2012). In that study, the observed reduction in incidence in cubs is reported as an indirect effect of vaccination. However, it is not possible to distinguish whether this reduction in incidence is due to the indirect effect achieved by a reduction in susceptibility or to a reduction in the infectivity of vaccinated infected badgers compared to non-vaccinated infected ones. Based on our results, the reduction in incidence among cubs was likely due to a reduction in susceptibility of the vaccinated adult badgers in the sett.

5. Conclusion

In summary, we have presented a new methodology to estimate both VE_S and VE_I providing further knowledge on the biological ways in which BCG vaccination works in badgers. We have also presented scientific arguments that support the crucial role of appropriate trial design in order to obtain accurate estimates. Further, we have estimated the impact of vaccination in the current badger transmission Ireland and concluded that a minimum vaccination coverage of 30% is necessary to achieve eradication of *M. bovis* infection in badgers. As a result of this work, policy makers can now make informed decisions concerning the best strategy or combination of strategies to achieve eradication. These results could also be used to guide the best vaccination route to achieve the minimum vaccine coverage needed.

Conflict of interest

No conflict of interest to declare

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.prevetmed.2017.10.010.

References

Ancelet, L.R., Aldwell, F.E., Rich, F.J., Kirman, J.R., 2012. Oral vaccination with lipid-formulated BCG induces a long-lived, multifunctional CD4(+) T cell memory immune response. PLoS One 7, e45888.

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., 2011. Trial design to estimate the effect of vaccination on tuberculosis incidence in badgers. Vet. Microbiol. 151, 104–111.

Aznar, I., More, S.J., Frankena, K., de Jong, M.C.M., 2013. Estimating the power of a *Mycobacterium bovis* vaccine trial in Irish badgers. Prev. Vet. Med. 111, 297–303.

Aznar, I., Frankena, K., More, S.J., Whelan, C., Martin, W., Gormley, E., Corner, L.A.L., Murphy, D., de Jong, M.C.M., 2014. Optimising and evaluating the characteristics of a multiple antigen ELISA for detection of Mycobacterium bovis infection in a badger vaccine field trial. PLoS One 9, e100139.

Bezos, J., Álvarez, J., Romero, B., de Juan, L., Domínguez, L., 2014. Bovine tuberculosis: historical perspective. Res. Vet. Sci. 97, S3–S4. http://dx.doi.org/10.1016/j.rvsc.2014.09.003. ISSN 0034-5288.

Byrne, A.W., Kenny, K., Fogarty, U., O'Keeffe, J.J., More, S.J., McGrath, G., Teeling, M., Martin, S.W., Dohoo, I.R., 2015. Spatial and temporal analysis of metrics of tuberculosis infection in badgers (*Meles meles*) from the Republic of Ireland: trends in apparent prevalence. Prev. Vet. Med. 122, 345–354.

Caminiti, A., Pelone, F., LaTorre, G., De Giusti, M., Saulle, R., Mannocci, A., Sala, M., Della Marta, U., Scaramozzino, P., 2016. Control and eradication of tuberculosis in cattle: a systematic review of economic evidence. Vet. Rec. 179, 70–75.

Carter, S.P., Chambers, M.A., Rushton, S.P., Shirley, M.D.F., Schuchert, P., Pietravalle, S., Murray, A., Rogers, F., Gettinby, G., Smith, G.C., Delahay, R.J., Hewinson, R.G., McDonald, R.A., 2012. BCG vaccination reduces risk of tuberculosis infection in vaccinated badgers and unvaccinated badger cubs. PLoS One 7, e49833.

Chambers, M.A., Crawshaw, T., Waterhouse, S., Delahay, R., Hewinson, R.G., Lyashchenko, K.P., 2008. Validation of the BrockTB Stat-Pak assay for detection of tuberculosis in Eurasian badgers (*Meles meles*) and influence of disease severity on diagnostic accuracy. J. Clin. Microbiol. 46, 1498–1500.

Chambers, M.A., Rogers, F., Delahay, R.J., Lesellier, S., Ashford, R., Dalley, D., Gowtage, S., Davé, D., Palmer, S., Brewer, J., Crawshaw, T., Clifton-Hadley, R., Carter, S., Cheeseman, C., Hanks, C., Murray, A., Palphramand, K., Pietravalle, S., Smith, G.C., Tomlinson, A., Walker, N.J., Wilson, G.J., Corner, L.A., Rushton, S.P., Shirley, M.D., Gettinby, G., McDonald, R.A., Hewinson, R.G., 2011. Bacillus Calmette-Guerin vaccination reduces the severity and progression of tuberculosis in badgers. Proc. R. Soc. B 278, 1913–1920.

Corner, L.A.L., O'Meara, D., Costello, E., Gormley, E., 2005. Tuberculosis in badgers: true prevalence, diagnostic methods and epidemiology (poster). Society for Veterinary Epidemiology and Preventive Medicine. . https://fr.slideserve.com/theodore-estes/tuberculosis-in-badgers-true-prevalence-diagnostic-methods-and-epidemiology Nairn, Scotland.

Corner, L.A.L., Costello, E., Lesellier, S., O'Meara, D., Gormley, E., 2008. Vaccination of European badgers (*Meles meles*) with BCG by the subcutaneous and mucosal routes induces protective immunity against endobronchial challenge with *Mycobacterium bovis*. Tuberculosis 88, 601–609.

Corner, L.A.L., Costello, E., O'Meara, D., Lesellier, S., Aldwell, F.E., 2010. Oral vaccination of badgers (*Meles meles*) with BCG and protective immunity against endobronchial challenge with *Mycobacterium bovis*. Vaccine 28, 6265–6272.

De Jong, M.C.M., Kimman, T.G., 1994. Experimental quantification of vaccine-induced reduction in virus transmission. Vaccine 12, 761–766.

Diekmann, O., Heesterbeek, J.A.P., Metz, J.A.J., 1990. On the definition and the computation of the basic reproduction ratio R_0 in models for infectious diseases in heterogeneous populations. J. Math. Biol. 28, 365–382.

Fine, P.E.M., 1993. Herd immunity: history, theory, practice. Epidemiol. Rev. 15, 265–302.

Gormley, E., Nı´ Bhuachalla, D., O'Keeffe, J., Murphy, D., Aldwell, F.E., Fitzsimons, T., Stanley, P., Tratalos, J.A., McGrath, G., Fogarty, N., Kenny, K., More, S.J., Messam, L.L., Corner, L.A.L., 2017. Oral vaccination of free-living badgers (*Meles meles*) with Bacille Calmette Guérin (BCG) vaccine confers protection against tuberculosis. PLoS One 12, e0168851.

Halloran Jr., M.E., Longini, I.M., Struchiner, C.J., 1999. Design and interpretation of vaccine field studies. Epidemiol. Rev. 21, 73–88.

Langer, A.J., LoBue, P.A., 2014. Public health significance of zoonotic tuberculosis caused by the Mycobacterium tuberculosis complex. In: Thoen, C.O., Steele, J.H., Kaneene, J.B. (Eds.), Zoonotic Tuberculosis: Mycobacterium Bovis and Other Pathogenic Mycobacteria, third edition. John Wiley & Sons, Inc., Chichester, UK.

Lesellier, S., Corner, L.A.L., Costello, E., Lyashchenko, K., Greenwald, R., Esfandiari, J., Singhd, M., Hewinsone, R.G., Chambers, M., Gormley, E., 2009. Immunological responses and protective immunity in BCG vaccinated badgers following endobronchial infection with *Mycobacterium bovis*. Vaccine 27, 402–409.

Lesellier, S., Palmer, S., Gowtage-Sequiera, S., Ashford, R., Dalley, D., Davé, D., Weyer, U., Salguero, F.J., Nunez, A., Crawshaw, T., Corner, L.A.L., Hewinson, R.G., Chambers, M.A., 2011. Protection of Eurasian badger (*Meles meles*) from tuberculosis after intra-muscular vaccination with different doses of BCG. Vaccine 29, 3782–3790.

Longini Jr., I.M., Sagatelian, K., Rida, W.N., Halloran, M.E., 1998. Optimal vaccine trial design when estimating vaccine efficacy for susceptibility and infectiousness from multiple populations. Stat. Med. 17, 1121–1136.

Moerman, A., Straver, P.J., de Jong, M.C., Quak, J., Baanvinger, T., van Oirschot, J.T., 1993. A long-term epidemiologic study of bovine viral diarrhea infections in a large herd of dairy cattle. Vet. Rec. 132, 622–626.

More, S.J., Good, M., 2006. The tuberculosis eradication programme in Ireland: a review of scientific and policy advances since 1988. Vet. Microbiol. 112, 239–251.

Murphy, D., Gormley, E., Costello, E., O'Meara, D., Corner, L.A.L., 2010. The prevalence and distribution of *Mycobacterium bovis* infection in European badgers (*Meles meles*) as determined by enhanced post mortem examination and bacteriological culture. Res. Vet. Sci. 88, 1–5.

Murphy, D., Costello, E., Aldwell, F.E., Lesellier, S., Chambers, M.A., Fitzsimons, T., Corner, L.A.L., Gormley, E., 2014. Oral vaccination of badgers (*Meles meles*) against tuberculosis: comparison of the protection generated by BCG vaccine strains Pasteur and Danish. Vet. J. 200, 362–367.

Stegeman, A., van Nes, A., de Jong, M.C.M., Bolder, F., 1995. Assessment of the effectiveness of vaccination against pseudorabies in finishing pigs. Am. J. Vet. Res. 56,

573–578.
Stephens, D.S., 2008. Vaccines for the unvaccinated: protecting the herd [Editorial commentary]. J. Infect. Dis. 197, 643–645.
Whelan, C., Shuralev, E., O'Keeffe, G., Hyland, P., Kwok, H.F., Snoddy, P., O'Brien, A., Connolly, M., Quinn, P., Groll, M., Watterson, T., Call, S., Kenny, K., Duignan, A., Hamilton, M.J., Buddle, B.M., Johnston, J.A., Davis, W.C., Olwill, S.A., Clarke, J., 2008. Multiplex immunoassay for serological diagnosis of *Mycobacterium bovis* infection in cattle. Clin. Vaccine Immunol. 15, 1834–1838.
Whelan, C., Whelan, A.O., Shuralev, E., Kwok, H.F., Hewinson, G., Clarke, J., Vordermeier, H.M., 2010. Performance of the Enferplex TB assay with cattle in Great Britain and assessment of its suitability as a test to distinguish infected and vaccinated animals. Clin. Vaccine Immunol. 17, 813–817.