Preventive Veterinary Medicine 2020; 183:105096, 1-7 (DOI: 10.1016/j.prevetmed.2020.105096)

Estimating wildlife vaccination coverage using genetic methods

Freya Smith[1], Andrew Robertson[2,1], Graham C. Smith[1], Peter Gill[3], Robbie A. McDonald[2], Gavin Wilson[4], Richard J. Delahay[1]

[1] National Wildlife Management Centre, Animal and Plant Health Agency, Woodchester Park, Gloucestershire, GL10 3UJ, UK.

[2] Environment and Sustainability Institute, University of Exeter, Penryn, Cornwall, TR10 9EZ, UK.

[3] Department of Forensic Biology, Oslo University Hospital and also Department of Forensic Medicine, University of Oslo, Oslo, Norway

[4] RSK Biocensus Limited, Suites 1-3 Bank House, Bond's Mill, Stonehouse, Gloucestershire, GL10 3RF, UK

Corresponding author: Freya.smith@apha.gov.uk

Key words: Wildlife, vaccination, vaccine coverage, capture mark recapture, genotyping, hair trap, badger, BCG, bovine tuberculosis

Abstract

Vaccination is a useful approach for the control of disease in wildlife populations. However, its effectiveness is dependent in part on delivery to a sufficient proportion of the target population. Measuring the proportions of wild animal populations that have been vaccinated is challenging and so there is a need to develop robust approaches that can contribute to our understanding of the likely efficacy of wildlife vaccination campaigns.

We used a modified capture mark recapture technique to estimate vaccine coverage in a wild population of European badgers (*Meles meles*) vaccinated by live-trapping and injecting with Bacillus Calmette-Guérin as part of a bovine tuberculosis control initiative in Wales, United Kingdom. Our approach used genetic matching of vaccinated animals to a sample of the wider population to estimate the percentage of badgers that had been vaccinated. Individual-specific genetic profiles were obtained using microsatellite genotyping of hair samples which were collected directly from trapped and vaccinated badgers and non-invasively from the wider population using hair traps deployed at badger burrows (setts).

With two nights of trapping at each sett in an annual campaign, an estimated 50% (95% confidence interval 40-60%) of the badger population received at least one dose of vaccine in a single year. Using a simple population model this suggested that the proportion of the population that would have received at least one dose of vaccine over the course of the four year vaccination campaign was between 67-83%.

This is the first attempt, outside of field trials, to quantify the level of vaccine coverage achieved by trapping and injecting badgers, which is currently the only option for delivering

44 BCG vaccine to this species. The results therefore have specific application to bTB control
45 policy and the novel approach may have wider value in wildlife management and research.
46

Introduction

Vaccination can contribute to disease control by reducing the number of susceptible and/or infectious individuals in a population and, thereby, the number of new infections. The approach has been applied to the control of reservoirs of wildlife disease (see Blancou et al., 2009). A particularly successful example has been the oral vaccination of red foxes (*Vulpes vulpes*), which resulted in the eradication of rabies from much of central and western Europe by the end of the 20th century (Muller et al., 2015). More recently, oral vaccination of wild boar has made a significant contribution to the control of Classical Swine Fever in parts of Europe (Rossi et al., 2015).

The effectiveness of vaccination as a means of disease control is influenced by several factors including disease prevalence, vaccine efficacy and, crucially, vaccine coverage in the target population. Attempts to estimate vaccine coverage in wild animals typically rely on post-vaccination surveillance in order to detect direct or indirect markers of vaccination in the target species (e.g. Rosatte et al., 2008; Rossi et al., 2015). This approach is usually dependent on the capture or recapture of vaccinated animals (demanding significant investment of time and resource), or the collection of carcasses (e.g. Johnston et al., 1988), which can be difficult to achieve.

Bovine tuberculosis (bTB) is a chronic disease of cattle caused by *Mycobacterium bovis*. The control of this infection in cattle, principally by test and slaughter based on the tuberculin skin test, has been successful at substantially reducing infection in cattle in several countries. However, in places where *M. bovis* persists in wildlife, disease control in cattle is more complex (Palmer et al., 2012). This is the case in the UK and Ireland, where

69 the European badger (*Meles meles*) is involved in the maintenance and transmission of
70 infection to cattle (Krebs et al., 1997; Bourne et al., 2007; Godfray et al., 2013).

71 Bacillus Calmette–Guérin (BCG) is an attenuated strain of *M. bovis* that has been used as
72 a live vaccine in badgers. When delivered by intramuscular injection, BCG has been
73 shown to slow the progression of disease, reducing both the severity of lesions and the
74 excretion of bacilli in experimentally challenged captive badgers (Chambers et al., 2011;
75 Lesellier et al., 2011). Field trials have also provided evidence of a protective effect in wild
76 badgers vaccinated with BCG by injection (Chambers et al., 2011; Carter et al., 2012), and
77 by oral administration (Gormley et al., 2017; Aznar *et al*., 2018). Furthermore, Carter et al.
78 (2012) inferred an indirect beneficial effect of vaccination, whereby unvaccinated cubs
79 born into badger social groups with a higher percentage of vaccinated adults were
80 significantly less likely to test positive for *M. bovis.* Together, these studies imply that
81 sustained vaccination could bring about a reduction in the prevalence of infection in
82 badgers (Chambers et al., 2014). As badgers contribute to infection in cattle, an effective
83 vaccination campaign would therefore be expected eventually to have a positive impact on
84 the control of disease in cattle, in line with predicted outcomes from simulation models
85 (Smith et al., 2012).

86 Although oral delivery of vaccine in bait may offer the most practical long term strategy for
87 widespread deployment of BCG in badgers (Blancou et al., 2009), such a formulation is
88 still in development and is unlikely to be available for some years to come (Chambers et
89 al., 2014). In the meantime an injectable BCG vaccine (BadgerBCG) has been licensed by
90 the UK Veterinary Medicines Directorate and is currently available for administration by
91 trapping and vaccinating badgers by intramuscular injection (Brown et al., 2013). Field
92 deployment of BadgerBCG has now been carried out at a number of locations across

England and Wales (APHA, 2015), during which badgers are live-captured, injected with the vaccine, temporarily marked (by clipping a patch of fur and applying stock-marker spray) and released. There are, however, no existing estimates of the level of vaccine coverage that has been achieved during these operations.

In order to estimate the proportion of the badger population captured and vaccinated (or captured for any other purpose), one requires an independent estimate of the wider population size, which can be challenging. Scheppers et al. (2007) devised a novel technique for estimating badger social group and population size by genotyping DNA from remotely plucked hairs, and this has subsequently been employed alongside surveys of setts (their underground burrows) to estimate badger abundance in England and Wales (Judge et al., 2017). In the current study we used an adapted version of this approach to estimate vaccine coverage during one year of a badger vaccination campaign in Wales, UK. We employed a genetic mark recapture approach to match hairs from vaccinated animals to those from a sample of the wider population. We then combined our results with estimates of population turnover in order to calculate the percentage of the total badger population that could be expected to have received at least one vaccine dose after the duration of the vaccination campaign, in this case four years.

Methods

Study area and population

The bTB Intensive Action Area (IAA) is a 288 km^2 area of high bTB incidence in cattle, located predominantly in north Pembrokeshire, Wales. The IAA has been subject to additional disease control measures over and above those in place in the rest of Wales, with the aim of reducing and eventually eliminating bTB in cattle in the area. The suite of measures applied to the IAA includes intensified cattle controls, heightened biosecurity measures, enhanced bovine TB testing regimes and badger vaccination (Welsh Government, 2016). Badger vaccination by live-trapping and injection was initiated in 2012 and repeated once per year for four years. In each of the first three years, over 1300 doses of BCG were administered annually to badgers from a population of unknown size (Welsh Government, 2016). The present study took place in the fourth and final year of the vaccination programme.

Badger trapping and vaccination

Badgers were captured using steel mesh traps, baited with peanuts and placed close to active badger setts or on active runs (visible paths created by habitual badger movements). All field operations were carried out by trained government operatives and licensable activities (the use of cage traps to trap and mark badgers) were authorised by the Welsh Government. Access for trapping was authorised by land owners for 249 km^2 covering 86% of the IAA (Welsh Government, 2016). Where access to setts or runs was not permitted, traps were located near linear features and boundaries (e.g. hedges) on adjacent accessible land. Following pre-baiting for approximately 7 days, traps were set for two consecutive nights (unless interrupted by adverse weather conditions). Captured

badgers were vaccinated by intra-muscular injection of 1 ml of BadgerBCG (2 to 8 x 10^6 colony forming units, BCG Danish strain 1331 vaccine, Statens Serum Institut, Copenhagen, Denmark), administered through the mesh of the trap. In order to avoid dosing animals more than once in the same year, vaccinated animals were temporarily marked by clipping the fur and applying a coloured stock marker prior to release. Vaccination was organised into seven cycles of three to four weeks duration, each targeting a different portion of the IAA and scheduled at approximately monthly intervals between May and October (Welsh Government, 2016). Hair trapping was staggered in line with vaccination cycles.

Genetic sampling

A tuft of approximately 5-10 guard hairs was removed by plucking from the rump of every trapped and vaccinated individual using artery forceps. Hairs were placed in a labelled sample bag together with a sachet of desiccant (Minipax ® absorbent packets, Sigma-Aldrich).

To remotely sample the wider badger population, we deployed ‘hair traps’ at a sub-sample of main setts following a methodology previously developed for estimating badger abundance (Frantz et al., 2004; Scheppers et al., 2007; Judge et al., 2017). Hair traps consisted of strands of barbed wire suspended across the sett entrance holes or on nearby badger runs. As the animals pass under the traps, their guard hairs catch on the wire barbs and can be collected for analysis.

Main setts were used as a proxy for badger social groups because there is usually only one main sett per group territory (Cresswell et al., 1990; Wilson et al., 1997). Main setts were distinguished from other less frequently used setts on the basis of the number of

active holes (Welsh Government 2016). Hair traps were deployed at a sample of 72 (28%) main setts from 260 identified during previous badger sett surveys of the IAA. Setts were selected by random sampling, stratified by scheduled vaccination month ('cycle'). Selected setts were revisited prior to setting hair traps, and those that were inactive, inaccessible or deemed not to be a main sett were substituted by randomly selected replacements from the same trap round. In total, 560 hair traps were deployed. Hair traps were placed to cover each active hole or badger run at each sett. Variation in sett size and numbers of active runs meant that the numbers of hair traps deployed differed amongst setts (minimum 2, maximum 18, median 8). Because we estimated coverage as the proportion of hair trapped individuals that were also vaccinated (instead of using hair trapping to arrive at a population estimate from which to derive coverage) the variation in hair trap number should not have biased our results.

For practical reasons, some of the hair traps remained *in situ* for more than 4 weeks. However, samples were only collected during a specified 28 day sampling period (Scheppers et al., 2007). All hair traps were cleaned of material (flamed) on day 0 of this 28 day period. On each visit, all the hairs on a given trap were removed and collected into a labelled bag containing a sachet of desiccant. If hair had caught on multiple barbs of the same trap, the hairs from each barb were collected into separate sample bags, which were then placed together within the same labelled bag. Each labelled bag therefore represented a specific hair trap on a given collection day. Once samples had been collected, hair traps were decontaminated by brief exposure to a naked flame using a gas lighter, in preparation for the next collection day.

Genetic typing

Hair samples were stored along with the desiccant sachet at 4°C shortly after collection. Up to 10 hairs were selected from every labelled sample bag, and pooled for genetic analysis. Each pool of hairs represented either an individual vaccinated animal or a specific hair trap-day combination. Hairs were selected on the basis of the size of the follicle as DNA recovery is generally more successful from larger follicles. In the case of the hair trap samples, all hairs selected originated from a single barb, with those from remaining barbs being retained for use if the profile from the original sample was suspected of being of mixed origin, see below.

DNA was extracted with a suspension of chelex resin (Frantz et al., 2004) using the Qiagen DNeasy® Blood and Tissue Kit. Genetic profiles were obtained by amplifying ten microsatellites (*Mel-103*, *Mel-104*, *Mel-105*, *Mel-107*, *Mel-110*, *Mel-113*, *Mel-114*, *Mel-115*, *Mel-116* and *Mel-117;* (Carpenter et al., 2003)). Microsatellite fragments were detected on an Applied Biosystems 3730xl Genetic Analyser and were analysed and sized using GeneMapper® Software (version 5).

Allele calling (designation of genotypes) was performed automatically using the Applied Biosystems GeneMapper® software. Each genotype was then reviewed manually by two operators. DNA profiles generated from the hair trap samples underwent a further manual review in order to screen for DNA profiles which contained contributions from more than one animal (mixed profiles). Suspected mixed profiles were identified on the basis of the presence of more than two alleles at one or more loci and/or a difference in peak height between heterozygous alleles such that a minimum threshold of heterozygote balance (the smallest allele in peak height divided by the largest allele in peak height) was exceeded at one or more loci (see S1 for further details). Suspected mixed profiles were excluded from further analyses in order to avoid artificially inflating the number of unique profiles obtained

204 from the background population. Where possible, we repeated extraction and genotyping
205 of these samples (this time, based on individual hairs rather than pooled hairs, if
206 necessary, from a different barb to the original sample analysed), although this was not
207 always feasible because the entire hair sample had often been used up in the first round of
208 analysis.

209 Genotype data were subsequently checked for the presence of null alleles (alleles which
210 failed to amplify reliably for a particular microsatellite) using the programme CERVUS
211 (Marshall et al., 1998). The output indicated that null alleles were present at microsatellite
212 *Mel-116* (null allele frequency = +0.2703). As a result, data associated with this
213 microsatellite were excluded from further analyses. Problems with this microsatellite are
214 similarly reported by Carpenter et al. (2005).

215 Trap sample matching

216 We used a modified version of the ‘cull sample matching' methodology developed to
217 estimate the effectiveness of badger culling using genetic samples (AHVLA 2014).
218 Genotypes derived from trapped and vaccinated badgers were matched (at the nine
219 remaining microsatellites) to those from the background (hair trapped) population using the
220 statistical package ALLELEMATCH (Galpern et al., 2012), executed in R 3.0.2 (R Development
221 Core Team, 2017). Incomplete profiles (due to failed amplification at one or more
222 microsatellites) were excluded from analyses. Two profiles were identified as being from the
223 same animal if they shared at least 17 of the 18 available alleles. Matching of profiles that
224 differ by one allele (or more in some cases), is commonly used in wildlife genetics studies
225 (e.g. Hettinga et al., 2012; Judge et al., 2017) where the quantity and quality of DNA may
226 be low, resulting in more frequent genotyping errors and hence a greater potential for
227 mismatching replicate samples. Initial analyses in the current study estimated that P_{sib} (the

probability that two samples could match at 17 alleles because they are siblings rather than duplicates) was <0.05 (mean=0.0017, min=0.0002, max=0.006), indicating that there was a very low possibility of matches between different individuals. However, the likelihood of false positives, false negatives, and other sources of error were further quantified following the approach set out in AHVLA (2014) and described below.

We calculated an initial estimate of vaccine coverage (percentage of badgers vaccinated) using the formula

$$\frac{x}{n} \times 100$$

where x is the number of hair trapped genotypes matching vaccinated badger genotypes and n is the number of hair trapped individuals (see also Figure 1). This initial estimate was then adjusted to account for the possibility of false positives (mistakenly matching samples originating from different individuals,FP) and false negatives (failure to match hair trap samples to vaccinated badger samples from the same individual, two sources considered, $FN1\ and\ FN2$) and to account for error associated with movement of animals within the population (FE).

Further detail on how these error rates were calculated is provided in the supplementary materials (S2). In brief, FP was the proportion of vaccinated badger genotypes that matched one another (under the assumption that badgers were only vaccinated once and therefore there should be no matches within this group), $FN1$ (failure to match hair trap samples from the same animal) was the proportion of hair trap genotypes which matched the same vaccinated badger, but which didn't match each other (based on a subset of the data which only included vaccinated badger genotypes matched to hair trap samples) and $FN2)$ (failure to match hair trap samples to vaccinated animals due to missing vaccinated badger

252 genotypes) was calculated as 1 minus the proportion of vaccinated badgers for which a
253 complete genotype was obtained. Finally, FE (error due to movement of animals into and
254 out of the study area) was estimated at 0-10% for animals from all social groups within 3 km
255 of the outer boundary of the IAA. This was based on the results of Rogers, Delahay et al.
256 (1998) who demonstrated, in a high density study population, that movement of badgers
257 between social groups occurred at up to 10% of trapping events, and that movements were
258 limited to 3 km (average 0.4-1 km). This work was carried out in an area dominated by Land
259 Class Group 4 (Bunce et al. 1981) which is also the predominant Land Class of the IAA.
260 Consequently, both study areas are expected to support similarly high density badger
261 populations (Judge et al. 2014, Judge et al. 2017). The distribution of each potential source
262 of error, was estimated as follows: FP, $FN1$, and $FN2$ were described by binomial distribution
263 with probability equal to FP, $FN1$, or $FN2$ (depending on the parameter being estimated) and
264 n equal to the sample size used to produce each error rate; FE was described by a uniform
265 distribution with a minimum of zero and a maximum equal to 0.1 (10%) multiplied by the
266 proportion of hair trap genotypes from social groups located within 3 km of the area
267 boundary. We then selected independent random values from binomial distributions as
268 realisations of the effect of FP on the number of hair trap samples matching vaccinated
269 badger genotypes, x (eFP, random binomial draw with size x and probability FP), and of the
270 effect of $FN1$, $FN2$ and FE on the background population, n ($eFN1$, $eFN2$, eFE, random
271 binomial draws with size n and probability $FN1$, $FN2$ and FE respectively). The adjusted
272 estimate of vaccine coverage was then calculated as a random quantile from the binomial
273 proportion below, converted to a percentage.

274 $$\frac{x - eFP}{n - eFE - eFN1 - eFN2} \times 100$$

This process was then repeated 1000 times, each time using a new draw from each of the error distributions (FP, $FN1$, $FN2$ and FE) and their corresponding realisations on x and n (eFP, $eFN1$, $eFN2$ and eFE) in order to calculate a mean estimate of adjusted vaccine coverage and its 95% confidence interval (2.5th and 97.5th percentiles). For more details see supplementary materials (S2).

Modelling cumulative vaccination coverage

Trap sample matching produced an estimate of vaccine coverage for a single year of vaccination. We used a simple quantitative population model to estimate the cumulative percentage of badgers likely to have received at least one vaccine dose by the end of a four year vaccination campaign. In a given year (t), the proportion of badgers in the standing population which had received at least one vaccine dose ($Vtotal_t$) was estimated as follows:

$$Vtotal_t = Vm_t + Vf_t + Vc_t + (Sm_t \times 1 - R) + (Sf_t \times 1 - R)$$

Where, Vm, Vf and Vc represent the proportion of adult male, adult female and cubs vaccinated in year t, Sm and Sf are the proportion of male and female vaccinated badgers surviving from previous years and R is the annual vaccine coverage. A step by step approach for solving this equation is provided in supplementary materials S3. It was not possible to estimate sex or age related variation in vaccine coverage using the methods outlined in this study, and therefore Vm, Vf and Vc were all equal. Parameters for demographic composition and associated survival rates (used to determine Sm and Sf) were fixed between years and based on published estimates from Smith et al. (2001), Table 1. Vaccination coverage was also assumed to be constant between years and was determined as outlined above (see results). We assumed a 50:50 sex ratio amongst cubs

298 (Roper 2010).For the purposes of the model the probability of capture was constant across
299 the population and between years Mean and upper and lower limits of our estimated
300 confidence interval for adjusted vaccine coverage (R) were used to calculate our mean
301 estimate of cumulative vaccination coverage ($Vtotal$) and the 95% confidence interval
302 around this estimate.

303

Results

Over the course of the year, a badger was trapped, vaccinated and its hair sampled on 1118 occasions (Figure 2). Of these hair samples, 1065 (95%) were successfully genotyped. Thirty-nine pairs of identical genotype profiles were identified amongst these hair samples and one genotype was present in triplicate. Field data showed that the samples from which the identical genotypes were derived were collected in relatively close proximity to one another (mean distance 397m, range 0-950m) but on different dates consistent with recapture and revaccination. Removal of these recaptures resulted in a final dataset of 1024 individual badgers trapped and vaccinated in the IAA.

In total, 682 hair trap-day samples were collected from 66 of 72 (92%) badger main setts (Figure 2), 384 (56%) of which yielded a complete genetic profile. Of these, 224 profiles (58%) were identified as single source (see S1) with the remaining 160 (42%) being potential mixtures, (i.e. possibly containing DNA from more than one animal). Reanalysis of single hairs from 78 of these 160 trap-day samples yielded a further 44 single source genetic profiles. Therefore, the total number of single source profiles from the background population was 268 (70% of all successfully profiled samples). Genotype matching indicated that these 268 profiles represented 141 unique individuals from 53 main setts. The number of unique individuals identified per sett (and hence per social group) ranged from 1 to 10 (median 2) (see S4).

Of the 141 unique individual profiles from hair traps, 68 (48%) matched those from a vaccinated badger hair sample (i.e. identical for at least 17 of 18 available alleles). This constituted our raw estimate of coverage.

326 Despite a low initial estimate of P_{sib}, there were 47 pairs of cage trapped badgers (total
327 1024) which were identical at 17 alleles, this is equivalent to a false positive rate (FP) of
328 0.046, or 4.6%. Of the 47 matches, 13 related to animals trapped on the same morning,
329 confirming that matches had occurred between different individuals. Realisations of the
330 effect of this error rate (eFP), indicated that between 0 and 7 matches (95% CI) were the
331 result of false positives. There were no instances of separate hair trapped samples
332 matching the same badger but failing to match each other ($FN1 = 0$). There were 49 hair
333 samples from cage trapped individuals which failed to produce a usable genotypes,
334 equivalent to a false negative rate ($FN2$) of 0.048 (4.8%). Realisations of the effect of this
335 error rate $eFN2$, indicated that between 2 and 12 (95% CI) hair trapped badgers could not
336 be matched on account of missing cage trap genotypes. Of the main setts producing hair
337 trapped genotypes, 66% were within 3km of the area boundary. Assuming a movement
338 rate of 0-10% at each of these setts, this means that 0.0-6.6% of badgers may have been
339 unavailable for sampling due to movement (FE = 0-0.066). Realisations of the effect of this
340 error rate $eFN2$, indicated that a further 0 to 11 (95% CI) hair trapped badgers were not
341 available for genetic matching, due to movements in/out of the area.

342 Accounting for errors (see S2 for more details), the adjusted estimate of coverage (the
343 percentage of badgers trapped and vaccinated) during the year of study was 50% of the
344 total population (95% confidence interval 40-60%). Assuming consistent annual coverage
345 at this level, our population model estimated that by the end of a four year vaccination
346 campaign 67-83% of the total population would have received at least one vaccine dose
347 (Figure 2).

348

Discussion

We employed a modified capture mark recapture method on genotypes obtained from hair samples to estimate population coverage for a vaccine delivered by trapping and injection. The approach taken has generic value for other applications in wildlife management and research where estimates of population size and the proportion that can be trapped or hair sampled would be useful, including studies aimed at measuring BCG uptake in other wildlife species implicated in *M. bovis* transmission (e.g. deer, wild boar, possums etc. see Palmer et al. (2012)). However, there are practical challenges with regard to remote hair sampling from animals with different ecological characteristics such that hair trapping technique (or other form of genetic sampling) would need to be adapted to the target species.

The present study has provided the first estimates of vaccine coverage for BCG administered to wild badgers in the UK, where vaccination by cage trapping and injection of BCG is currently carried out under licence. This is a valuable piece of information in the assessment of the cost-effectiveness of this tool for reducing bTB risks to cattle.

Our results suggest that in a single year of vaccination, 50% (95% confidence interval 40-60%) of badgers in the IAA were trapped and vaccinated. Assuming equivalent levels of coverage were achieved in preceding years, we estimate that by the end of four consecutive years of vaccination, 67-83% of the total badger population in the IAA would have received at least one dose of vaccine. As BCG does not provide complete immunity in badgers, our estimate of vaccine coverage should not be interpreted as indicative of protection at the population level. Rather it is a measure of the proportion of the population to which vaccine can be successfully delivered. Furthermore, our estimates are clearly

372 specific to this study. We might expect, for example, a different outcome from vaccination
373 operations in lower density badger populations or by programmes relying more heavily on
374 remote trapping. That said, despite using a different approach (with respect to both
375 trapping for vaccination and estimation of coverage) Byrne et al. (2012) generated a
376 similar estimate of vaccine coverage (79% adults) in a low density population in Ireland.
377 The model we used to estimate cumulative vaccination coverage assumed that capture
378 probability was equal across the population and constant between years. Models are, by
379 definition a simplification of a real world scenario. In reality capture probability is likely to
380 vary between individuals (e.g. according to sex, age, TB infection status) and potentially
381 also through time. Failing to account for this variation could have led to overestimation of
382 cumulative vaccination coverage (e.g. if there is a tendency for some animals to be more
383 trap-happy than others, or in the event that trapability increases over the lifetime of a
384 project due to badgers becoming accustomed to taking bait).

385 An important assumption underlying all capture mark recapture methodologies is that the
386 'marked' sample of animals is representative of the target or wider, background population.
387 In the present study, the marked sample was established using remote sampling of hairs
388 and genotyping. This approach has an advantage over conventional capture mark
389 recapture methods because it means that sampling of the background population is not
390 dependent on trapping animals and so is independent of the method used to sample the
391 vaccinated population. As a result, our wider, hair-trapped sample is likely to have included
392 any trap-shy animals and is therefore likely to be more representative of the background
393 population. In order to further satisfy the assumption of representative sampling, the setts
394 included in this study were randomly selected, and sample size was high (we deployed
395 hair traps at more than a quarter of all main setts in the IAA). We also collected samples

396 over a relatively long time period (alternate days for a four week period at each site (see
397 Scheppers et al. 2007) in order to sample animals that were not permanently resident at
398 the main sett. However, sampling bias in favour of animals that spent more time at main
399 setts than at outlier setts, cannot be ruled out. If such animals were also more likely to be
400 trapped for vaccination then this could have resulted in an overestimate of coverage. On
401 the other hand, it is possible that some of the remotely trapped hairs were from non-
402 resident animals, in which case this could have resulted in an underestimate of coverage.
403 There is also a chance that sampling was biased towards adult badgers, as small cubs
404 may have passed beneath hair traps without coming into contact with the barbed wire.
405 However as remote hair trapping did not commence until June it is unlikely that many (if
406 any) sufficiently small cubs would have been present (Roper, 2010).

407 There are several sources of genotyping error which could potentially have impacted our
408 results (reviewed by Pompanon et al., 2005). We mitigated the risk of false negative
409 matches (failure to match samples from the same animal) by matching at 17 alleles rather
410 than the full complement of 18. This resulted in a number of presumed false matches
411 between different vaccinated animals, but this source of error was quantified and
412 incorporated into our calculations and so should not have biased our final estimate of
413 coverage. Also, genotyping of hair trapped samples from pooled hairs could have
414 contributed to mismatching by creating false genotypes within the background population
415 (mixed DNA profiles). To minimise this potential source of error, all DNA profiles derived
416 from pooled hair trapped samples were screened for markers of mixed DNA prior to being
417 matched with vaccinated animals. Our protocol for screening was conservative (see S1),
418 such that we almost certainly rejected some single source profiles, thus reducing the
419 overall sample size (for more than 50% of the profiles identified as being ‘mixed’, the entire

sample was used up in the first round of genotyping and hence no hair was available for retesting). However, although this may have increased uncertainty in our estimate of coverage, because these individuals should be random in relation to their likelihood of being captured, it should not have introduced bias.

Trapping and injecting animals remains the only approach for BCG administration in wild badgers in the UK that is currently available (Chambers 2014). An alternative approach might involve delivery of an oral vaccine in a bait that could be deployed at setts (Delahay et al., 2003). In such circumstances the approach described in the present study could be adapted to monitor vaccine coverage, if the bait also included a marker that could be detected in hair samples.

Conclusion

The present study is the first to estimate vaccine coverage in a UK badger population subjected to cage trapping and injection. We have demonstrated that even at the lower estimate of annual vaccine coverage observed, it may be possible to vaccinate 40% (mean 50%, 95% confidence interval 40-60%) of the badger population with two nights' trapping in a given year, and that this is consistent with achieving 70% coverage by the end of a four year annual vaccination campaign. This is encouraging as estimates of R0 (the number of secondary infections per individual in a naïve population) for bTB in badgers are generally low (Smith, 2001; Cox et al., 2005; Delahay et al., 2013), implying that relatively low levels of vaccination may be sufficient to drive the disease to extinction, given sufficient time. Consistent with this view, Aznar et al. (2018) recently estimated that in Ireland, 30% vaccine coverage (based on oral vaccine administration) would be sufficient to reduce the effective reproductive number in badgers below one. The current

study suggests that vaccine coverage of at least this magnitude can be achieved, even when only trapping at each sett for two nights once a year to deliver the injectable vaccine.

Acknowledgements

This study was funded by the Welsh Government. We are indebted to our colleagues in the Welsh Government Office of the Chief Veterinary Officer for their assistance in coordinating the project, and to the OCVO and APHA field staff for carrying out the field work. We are also grateful to Saira Cawthraw and other staff at the APHA Central Sequencing Laboratory for expert technical assistance, and to Bronwen Daniels and Rowena Staff of the NWMC for project support. Finally, we are indebted to the landowners and tenants in the Intensive Action Area for granting access to their land.

References

AHVLA (2014). The efficacy of badger population reduction by controlled shooting and cage trapping, and the change in badger activity following culling from 27/08/2013 to 28/11/2013. Report to Defra. https://assets.publishing.service.gov.uk/government/uploads/system/uploads/attachment_data/file/300385/ahvla-extension-efficacy.pdf (accessed 7 March 2019)

APHA, 2015. Badger Vaccine Deployment Project: lessons learned report. https://www.gov.uk/government/publications/badger-vaccine-deployment-project-lesson-learned-report (accessed 7 March 2019).

Aznar, I., Frankena, K., More, S., O'Keeffe, J., McGrath, G., de Jong, M., 2018. Quantification of Mycobacterium bovis transmission in a badger vaccine field trial. Prev. Vet. Med. 149, 29-37. https://assets.publishing.service.gov.uk/government/uploads/system/uploads/attachment_data/file/300385/ahvla-extension-efficacy.pdf (acessed 7 March 2019).

Blancou, J., Artois, M., Gilot-Fromont, E., Kaden, V., Rossi, S., Smith, G.C., Hutchings, M.R., Chambers, M.A., Houghton, S., Delahay, R.J., 2009. Options for the control of disease 1: targeting the infectious or parasitic agent. In: Delahay, R., Smith, G.C., Hutchings, M.R. (Eds.), Management of Disease in Wild Mammals. Springer, 97-120.

Bourne, F.J., Donnelly, C.A., Cox, D.R., Gettinby, G., McInerney, J., Morrison, I., Woodroffe, R., 2007. Bovine TB: the scientific evidence. Final report of the independent scientific group on cattle TB. Defra, London, UK.

478 Brown, E., Cooney, R., Rogers, F., 2013. Veterinary guidance on the practical use of the
479 BadgerBCG tuberculosis vaccine. In Practice 35, 143-146.

480 Bunce, R. G. H., C. Barr and H. Whittaker (1981). Land classes in Great Britain:
481 preliminary descriptions for users of the Merlewood method of land classification.

482 Byrne, A. W., J. O'Keeffe, S. Green, D. P. Sleeman, L. A. Corner, E. Gormley, D. Murphy,
483 S. W. Martin and J. Davenport (2012). "Population estimation and trappability of the
484 European badger (Meles meles): implications for tuberculosis management." PloS
485 one **7**(12).

486 Carpenter, P.J., Dawson, D.A., Greig, C., Parham, A., Cheeseman, C.L., Burke, T., 2003.
487 Isolation of 39 polymorphic microsatellite loci and the development of a
488 fluorescently labelled marker set for the Eurasian badger (Meles meles). Mol. Ecol.
489 Notes 3, 610-615.

490 Carpenter, P. J., L. C. Pope, C. Greig, D. A. Dawson, L. M. Rogers, K. Erven, G. J. Wilson,
491 R. J. Delahay, C. L. Cheeseman and T. Burke (2005). Mating system of the
492 Eurasian badger, Meles meles, in a high density population. Mol. Ecol. 14(1): 273-
493 284.

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

Carter, S.P., Robertson, A., Palphramand, K.L., Chambers, M.A., McDonald, R.A., Delahay, R.J., 2018. Bait uptake by wild badgers and its implications for oral vaccination against tuberculosis. PloS One 13, e0206136.

Chambers, M.A., Aldwell, F., Williams, G.A., Palmer, S., Gowtage, S., Ashford, R., Dalley, D.J., Davé, D., Weyer, U., Salguero, F.J., 2017. The effect of oral vaccination with Mycobacterium bovis BCG on the development of tuberculosis in captive European badgers (Meles meles). Front. Cell. Infect. Microbiol 7, 6.

Chambers, M.A., Carter, S.P., Wilson, G.J., Jones, G., Brown, E., Hewinson, R.G., Vordermeier, M., 2014. Vaccination against tuberculosis in badgers and cattle: an overview of the challenges, developments and current research priorities in Great Britain. Vet. Rec. 175, 90-96.

Chambers, M.A., Rogers, F., Delahay, R.J., Lesellier, S., Ashford, R., Dalley, D., Gowtage, S., Dave, 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. Lond., Ser. B: Biol. Sci. 278, 1913-1920.

Cox, D., Donnelly, C.A., Bourne, F.J., Gettinby, G., McInerney, J.P., Morrison, W.I., Woodroffe, R., 2005. Simple model for tuberculosis in cattle and badgers. Proceedings of the National Academy of Sciences 102, 17588-17593.

Cresswell, P., Harris, S., Jefferies, D.J., 1990. The history, distribution, status and habitat requirements of the badger in Britain. Nature Conservancy Council Peterborough.

521 Defra, 2010. Badger vaccine study. Field trial to assess the safety and efficacy of Bacille
522 Calmette Guérin (BCG) vaccine administered parenterally to badgers (Good Clinical
523 Practice [veterinary] study on wild badgers). Final Study Report VLAS/05/036.
524 http://www.bovinetb.info/docs/field-trial-to-assess-the-safety-of-bacille-calmette-
525 guerin-vaccine-administered-parenterally-to-badgers.pdf (accessed 7 March 2019)

526 Delahay, R.J., Walker, N., Smith, G.S., Wilkinson, D., Clifton-Hadley, R.S., Cheeseman,
527 C.L., Tomlinson, A.J., Chambers, M.A., 2013. Long-term temporal trends and
528 estimated transmission rates for Mycobacterium bovis infection in an undisturbed
529 high-density badger (Meles meles) population. Epidemiol. Infect. 141, 1445-1456.

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

531 Frantz, A.C., Schaul, M., Pope, L.C., Fack, F., Schley, L., Muller, C.P., Roper, T.J., 2004.
532 Estimating population size by genotyping remotely plucked hair: the Eurasian
533 badger. J. Appl. Ecol. 41, 985-995.

534 Galpern, P., Manseau, M., Hettinga, P., Smith, K., Wilson, P., 2012. Allelematch: an R
535 package for identifying unique multilocus genotypes where genotyping error and
536 missing data may be present. Mol. Ecol. Resour. 12, 771-778.

537 Godfray, H.C., Donnelly, C.A., Kao, R.R., Macdonald, D.W., McDonald, R.A., Petrokofsky,
538 G., Wood, J.L., Woodroffe, R., Young, D.B., McLean, A.R., 2013. A restatement of
539 the natural science evidence base relevant to the control of bovine tuberculosis in
540 Great Britain. Proc. R. Soc. Lond., Ser. B: Biol. Sci. 280, 20131634.

541 Gormley, E., Bhuachalla, D.N., O'Keeffe, J., Murphy, D., Aldwell, F.E., Fitzsimons, T.,
542 Stanley, P., Tratalos, J.A., McGrath, G., Fogarty, N., 2017. Oral vaccination of free-

543 living badgers (Meles meles) with Bacille Calmette Guerin (BCG) vaccine confers
544 protection against tuberculosis. PloS One 12, e0168851.

545 Gowtage, S., Williams, G.A., Henderson, R., Aylett, P., MacMorran, D., Palmer, S.,
546 Robertson, A., Lesellier, S., Carter, S.P., Chambers, M.A., 2017. Testing of a
547 palatable bait and compatible vaccine carrier for the oral vaccination of European
548 badgers (Meles meles) against tuberculosis. Vaccine 35, 987-992.

549 Griffin, J., Mackintosh, C., Slobbe, L., Thomson, A., Buchan, G., 1999. Vaccine protocols
550 to optimise the protective efficacy of BCG. Tubercle Lung Dis. 79, 135-143.

551 Hettinga, P.N., Arnason, A.N., Manseau, M., Cross, D., Whaley, K., Wilson, P.J., 2012.
552 Estimating size and trend of the North Interlake woodland caribou population using
553 fecal-DNA and capture–recapture models. J. Wildl. Manage. 76, 1153-1164.

554 Johnston, D.H., Voigt, D.R., MacInnes, C.D., Bachmann, P., Lawson, K.F., Rupprecht,
555 C.E., 1988. An aerial baiting system for the distribution of attenuated or
556 recombinant rabies vaccines for foxes, raccoons, and skunks. J. Infect. Dis. 10,
557 S660-S664.

558 Judge, J., Wilson, G.J., Macarthur, R., McDonald, R.A., Delahay, R.J., 2017. Abundance
559 of badgers (Meles meles) in England and Wales. Sci. Rep. 7, 276.

560 Judge, J., G. J. Wilson, R. Macarthur, R. J. Delahay and R. A. McDonald (2014). Density
561 and abundance of badger social groups in England and Wales in 2011-2013 Sci.
562 Rep. 4, 3809.

563 Krebs, J.R., Anderson, R.M., Clutton-Brock, T., Morrison, I., Young, D.B., Donnelly, C.A.,
564 1997. Bovine tuberculosis in cattle and badgers. Defra, London, UK.

565 Lesellier, S., Palmer, S., Gowtage-Sequiera, S., Ashford, R., Dalley, D., Dave, D., Weyer,
566 U., Salguero, F.J., Nunez, A., Crawshaw, T., Corner, L.A.L., Hewinson, R.G.,
567 Chambers, M.A., 2011. Protection of Eurasian badgers (Meles meles) from
568 tuberculosis after intra-muscular vaccination with different doses of BCG. Vaccine
569 29, 3782-3790.

570 Marshall, T.C., Slate, J., Kruuk, L.E.B., Pemberton, J.M., 1998. Statistical confidence for
571 likelihood-based paternity inference in natural populations. Mol. Ecol. 7, 639-655.

572 Muller, T.F., Schroder, R., Wysocki, P., Mettenleiter, T.C., Freuling, C.M., 2015. Spatio-
573 temporal Use of Oral Rabies Vaccines in Fox Rabies Elimination Programmes in
574 Europe. PLOS Negl. Trop. Dis. 9, e0003953.

575 Palmer, M.V., Thacker, T.C., Waters, W., Gortázar, C., Corner, L.A., 2012. Mycobacterium
576 bovis: a model pathogen at the interface of livestock, wildlife, and humans.
577 Veterinary Medicine International 2012.

578 Palphramand, K., Delahay, R., Robertson, A., Gowtage, S., Williams, G.A., McDonald,
579 R.A., Chambers, M., Carter, S.P., 2017. Field evaluation of candidate baits for oral
580 delivery of BCG vaccine to European badgers, Meles meles. Vaccine 35, 4402-
581 4407.

582 Parlane, N.A., Shu, D., Subharat, S., Wedlock, D.N., Rehm, B.H., de Lisle, G.W., Buddle,
583 B.M., 2014. Revaccination of cattle with bacille Calmette-Guerin two years after first
584 vaccination when immunity has waned, boosted protection against challenge with
585 Mycobacterium bovis. PloS One 9, e106519.

586 Pompanon, F., Bonin, A., Bellemain, E., Taberlet, P., 2005. Genotyping errors: causes,
587 consequences and solutions. Nat. Rev. Genet. 6, 847-846.

588 R Development Core Team, 2017. R: A language and environment for statistical
589 computing. R Foundation for Statistical Computing, Vienna, Austria.

590 Robertson, A., Delahay, R.J., McDonald, R.A., Aylett, P., Henderson, R., Gowtage, S.,
591 Chambers, M.A., Carter, S.P., 2016. Behaviour of European badgers and non-
592 target species towards candidate baits for oral delivery of a tuberculosis vaccine.
593 Prev. Vet. Med. 135, 95-101.

594 Roper, T.J., 2010. Badger. Collins London.

595 Rosatte, R., Allan, M., Bachmann, P., Sobey, K., Donovan, D., Davies, J., Silver, A.,
596 Bennett, K., Brown, L., Stevenson, B., 2008. Prevalence of tetracycline and rabies
597 virus antibody in raccoons, skunks, and foxes following aerial distribution of V-RG
598 baits to control raccoon rabies in Ontario, Canada. J. Wildl. Dis. 44, 946-964.

599 Rossi, S., Staubach, C., Blome, S., Guberti, V., Thulke, H.H., Vos, A., Koenen, F., Le
600 Potier, M.F., 2015. Controlling of CSFV in European wild boar using oral
601 vaccination: a review. Front. Biol. 6, 1141.

602 Scheppers, T.L.J., Frantz, A.C., CSchaul, M., Engel, E., Breyne, P., Schley, L., Roper,
603 T.J., 2007. Estimating social group size of Eurasian badgers *Meles meles* by
604 genotyping remotely plucked single hairs. Wildl. Biol. 13.

605 Smith, G., 2001. Models of Mycobacterium bovis in wildlife and cattle. Tuberculosis 81, 51-
606 64.

607 Smith, G.C., McDonald, R.A., Wilkinson, D., 2012. Comparing badger (Meles meles)
608 management strategies for reducing tuberculosis incidence in cattle. PloS One 7,
609 e39250.

610 Welsh Government, 2016. Bovine TB Eradication Programme IAA Vaccination Project -
611 Year 4 Report. http://www.bovinetb.info/docs/bovine-tb-eradication-programme-iaa-
612 vaccination-project-year-4-report.pdf (accessed 7 March 2019).

613 Wilson, G., Harris, S., McLaren, G., 1997. Changes in the British badger population, 1988
614 to 1997 People's Trust for Endangered Species London.

615

Figures

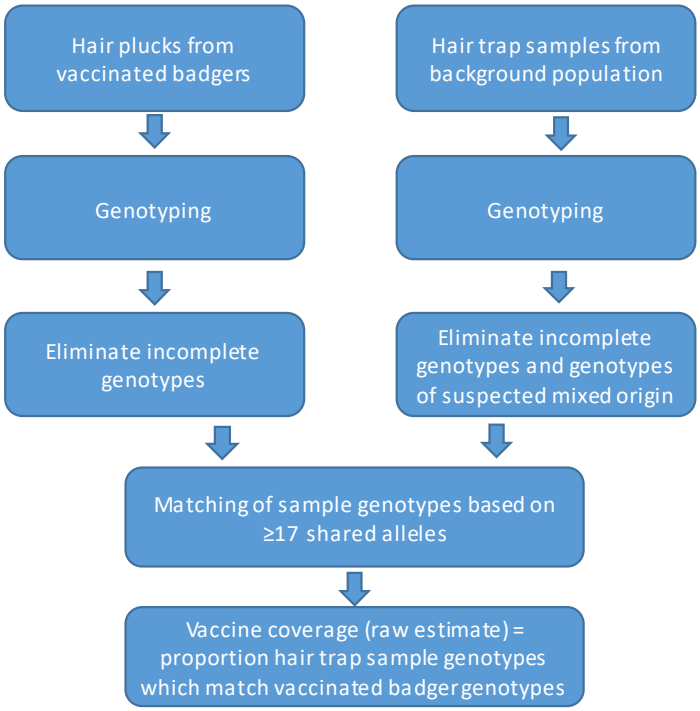
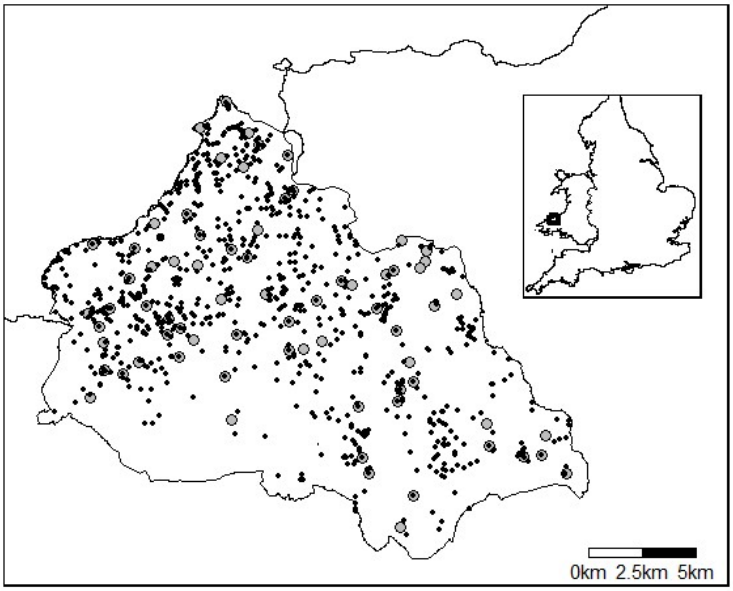


Figure 1 Schematic outlining the key stages involved in generating a raw estimate of vaccine coverage

620

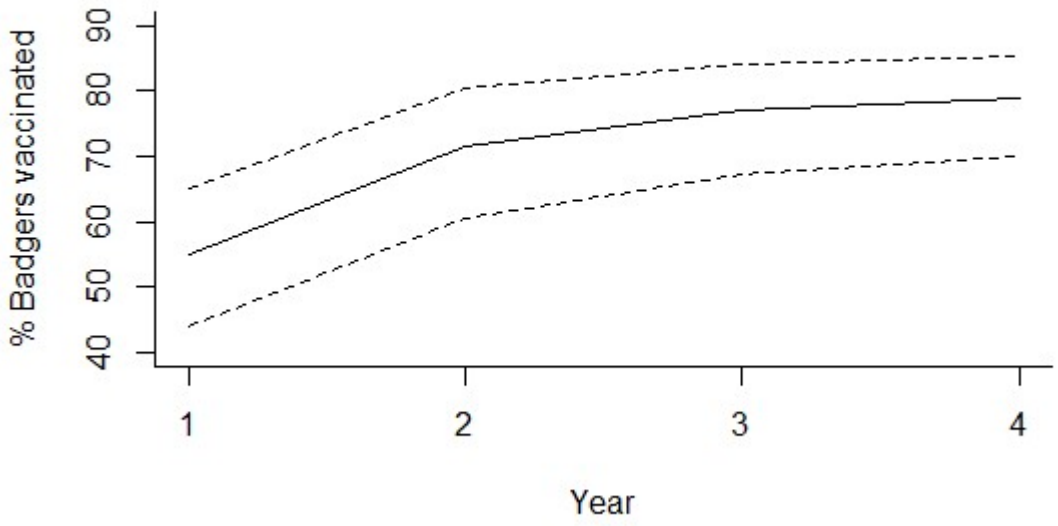


621

622 Figure 2 Map of the Intensive Action Area for bovine tuberculosis control. Black dots show locations of
623 trapped and vaccinated badgers. Grey circles show locations of hair trapped setts.

624

625



626

627 Figure 3. Modelled 95% confidence interval for the percentage of badgers expected to have received at least
628 one vaccine dose by the end of a four year vaccination campaign.

629

Tables

Table 1 Parameters used to estimate cumulative vaccination coverage. The demographic parameters were adapted from Smith et al. (2001). The vaccination parameter was derived from the results of the current study.

Parameter	Description	Value
R	% badgers vaccinated	mean 50%, 95% CI 40-60%
Q_m	Annual survival probability of adult male badgers	0.66
Q_f	Annual survival probability of adult female badgers	0.75
Q_c	Annual survival probability of badger cubs	0.6
M	Adult male badger population (%)	28.65
F	Adult female population (%)	38.90
C	Cub population (%)	32.45