Estimating the population size and the percentage of vaccinated badgers in the Intensive Action Area

Report to the Welsh Government

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Animal &
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1. Executive summary

1.1. The Intensive Action Area (IAA), established in 2010, is a 288 km^2 area primarily located in north Pembrokeshire where increased control measures are implemented to tackle all sources of bovine TB in cattle.

1.2. One feature of the IAA since 2012 has been the annual vaccination of badgers for a planned five year period.

1.3. In 2015 (the fourth year of the vaccination project) the Welsh Government commissioned a study to estimate the size of the badger population in the IAA and the percentage of the population that was vaccinated in 2015.

1.4. We used an established method (trap sample matching) to compare the genetic profiles of all vaccinated badgers with those of animals whose hairs were collected from barbed wire hair traps deployed at a random sample of main setts in the IAA. The percentage of the total badger population that had received a vaccination in 2015 was estimated as the percentage of hair trapped individuals that matched the profile of vaccinated badgers.

1.5. Population size was calculated as the total number of vaccinated badgers, divided by the estimated percentage of the population that had received a vaccination in 2015.

1.6. Vaccination in the IAA took place between May and October 2015. Hairs were collected for genotyping from 1118 vaccinated badgers. In total 95% of hair samples from vaccinated badgers yielded useable genotype data.

1.7. Hair trapping took place between June and December 2015. In total 560 hair traps were deployed at 72 main setts (ranging from 2 to 18 traps per sett) returning 682 unique trap-day samples. In total 39% of hair trap samples yielded useable genotype data.

1.8. Based on matching genotypes from hair traps and vaccinated badgers, the percentage of the badger population in the IAA that was vaccinated in 2015 was estimated to be between 44-65% (95% confidence interval).

1.9. The total badger population size in the IAA was estimated to be between 1645-2457 badgers (95% confidence interval), equivalent to a density of 5.7-8.5 badgers per km^2.

1.10. Based on the results of the present study, population modelling estimated that 70-85% of the total population could have received at least one vaccine dose by the end of a four year vaccination campaign, assuming a constant level of annual coverage consistent with that observed in 2015.

1.11. This study represents the first attempt to estimate the percentage of badgers vaccinated by trapping and injection. The results suggest that even at the lower annual estimate of the percentage trapped and vaccinated, it could be possible to achieve cumulative vaccination coverage of 70% over the course of a four year vaccination programme.

2. Introduction

The bovine TB Intensive Action Area (IAA) is a 288 km^2 area primarily located in north Pembrokeshire, Wales (Figure 1). The IAA has one of the highest incidences of bovine TB in Wales. Since 2010, the IAA has been subject to additional disease control measures over and above those in place across the rest of the country, with the aim of reducing and eventually eradicating TB in cattle in the area. The additional measures include stricter cattle controls, improved biosecurity and compulsory TB testing of goats and camelids.

In March 2012 it was announced that the Welsh Government would also undertake a badger vaccination project within the IAA. Vaccination is conducted by trapping badgers in steel mesh box traps in the vicinity of their setts and administering an intra-muscular injection of Badger BCG© before release back into the wild. During the first 3 years of the project (2012-2014) over 1300 vaccinations were deployed annually (in excess of 4000 vaccine doses in total).

Figure 1 Map of the Intensive Action Area

At a population level, the success of any vaccination campaign is a function of the efficacy of the vaccine and the scale of coverage achieved. Although some information exists on the efficacy of BCG vaccination of badgers (Lesellier *et al.* 2006, Lesellier *et al.* 2011, Carter *et al.* 2012), it is not known what percentage of a wild badger population could typically be trapped and vaccinated.

Previous studies have shown that sufficient genetic information can be derived from badger hair samples to identify different individuals (Frantz *et al.* 2004). Badger hair

samples can be collected directly from trapped animals or remotely by using hair-trapping devices deployed at badger setts. The genotyping of hairs collected by these methods has been used to generate estimates of the percentage of badgers removed during industry-led badger culling in 2013 in England (AHVLA 2014). In the current study, we used the same 'trap sample matching' approach to estimate the total badger population size in the IAA and the percentage of badgers that had been trapped and vaccinated during 2015.

3. Methods

3.1. General approach

Trap sample matching involves matching trapped and vaccinated individuals to a representative sample of the background population on the basis of their genetic identities. The background sample is established by genotyping hairs captured using barbed wire hair traps deployed at a random sample of main setts (see section 3.2). The vaccinated population is established by genotyping hairs plucked from every vaccinated badger (see section 3.4). The percentage of individuals within the background sample that are also cage trapped and vaccinated is an estimate of the percentage of the total badger population vaccinated.

3.2. Hair trapping

As in previous studies (e.g. Wilson *et al.* 1997, Judge *et al.* 2014) the main sett was used as a proxy for badger social group. There is typically only one main sett per territory and this forms the focus of breeding and social activity for most individuals within the group (Roper 2010).

Hair traps were laid at 72 active main setts selected randomly from the 260 identified during sett surveys undertaken by Welsh Government employees in 2013. The sample of 72 setts represents approximately 28% of the total number. Sett data from 2014 could not be used due to a change in main sett classification during that year.

The status of each main sett was verified by experienced APHA field workers from the National Wildlife Management Centre prior to any hair traps being deployed. Sett classification was evaluated on the basis of size (number of entrance holes) and the presence of fresh field signs. Any sett that did not qualify as a main sett, or where access was refused, was substituted by a randomly selected replacement.

Hair traps consisted of strands of barbed wire suspended across sett entrances and/or nearby badger runs, using natural features where possible or wooden stakes if necessary. Each trap was labelled with a unique identifier. Hair traps remained in situ for at least 4 weeks and were visited on alternate days over a 28 day sampling period following the methods of Frantz *et al.* (2004) and Scheppers *et al.* (2007). On each visit all the hairs on a given hair trap were collected into a barcoded bag together with a sachet of desiccant. If hairs had caught on multiple barbs, separate small bags were used for each barb. All small bags were then enclosed in a single barcoded bag. The sample of hairs in each barcoded

bag therefore represented a specific hair trap-day combination. Once samples had been collected, hair traps were decontaminated by brief exposure to a naked flame.

3.3. Vaccination

Vaccination of badgers in the IAA was carried out by Welsh Government field staff in accordance with the Protection of Badgers Act 1992 and Wildlife and Countryside Act 1981, and under the direction of Welsh Government veterinary surgeons. All field operatives who vaccinated badgers had successfully completed the APHA Cage Trapping and Vaccination of Badgers Course.

Vaccination was undertaken in work cycles each lasting for three to four weeks. A work cycle began with a visit to the landowner to request permission for access to survey the land. The next stage involved surveying for badger activity and positioning of cage traps. Traps were then tied open and pre-baited with peanuts for a period of time to allow the badgers to become familiar with them. Finally, in the last week of the cycle the traps were set to catch and any badgers caught in the traps were vaccinated. Trapped badgers were vaccinated by intra-muscular injection of BCG and temporarily marked by clipping the fur and applying coloured stock marker. Any trapped animals that had previously been marked were recorded as recaptures and released without vaccination.

3.4. Hair sampling of vaccinated badgers

A pluck of approximately 5-10 guard hairs was taken from the rump of every vaccinated badger. The hair pluck was carried out immediately after vaccination and was only performed by trained vaccinators. A pair of artery forceps was used to lock around the selected hairs which were then removed by pulling sharply away from the animal. Where necessary, wickets were used to momentarily restrain the badger. Each pluck was then collected into a barcoded bag together with a sachet of dessicant.

3.5. Genotyping

DNA was extracted from hairs using a suspension of chelex resin (Frantz *et al.* 2004) using the Qiagen DNeasy® Blood and Tissue Kit. Where possible, ten hairs were selected from each barcoded sample bag (i.e. a single trap-date combination for hair trap samples or pluck from an individual vaccinated badger). For hair trap samples, hairs from different barbs of the same trap were collected into individual small sample bags all contained within a single barcoded bag. In this case, all hairs selected for analysis were taken from the same small bag representing a single barb. Selection of hairs was based on the size of the hair follicle, which is the source of the DNA. Where fewer than ten hairs were available, all hairs in a bag were selected.

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). Incomplete profiles (where amplification had failed at one or more loci) were excluded from the analyses.

3.6. Data quality control

Automated allele calls performed in GeneMapper® were checked by two operators. This involved visual inspection of sample electropherograms (the graphical representation of amplified DNA used for determining genotype, EPG). Final allele designation was determined by consensus between operators.

All profiles derived from vaccinated badgers were assumed to represent individual animals as the risk of contamination between hair plucks obtained directly from vaccinated badgers was considered to be very low. On the other hand, it was considered likely that a proportion of the hair trap samples would contain contributions from more than one animal and if these individuals were represented in the hairs selected for analysis, this could result in a mixed genetic profile. It was important to identify and remove these mixed profiles prior to analysis as failure to do so could artificially inflate the estimated population size and consequently, result in an underestimate of the percentage of vaccinated animals. Identification of mixed profiles required further visual inspection of hair trap EPGs to identify instances where (1) three or more alleles were present at one or more loci and/or (2) the difference in allele height at heterozygous loci exceeded a predetermined threshold. Details of the methods used to identify mixed profiles can be found in Appendix A. In cases where a profile was identified as mixed, we first checked to see whether there were any unprocessed hairs for the specific trap-date combination. If hairs were remaining, includings hairs collected from a different barb of the trap, a single hair was selected for analysis following the methods described above.

3.7. Identification of null alleles

Genotype data were checked for the presence of null alleles (alleles which failed to amplify reliably for a particular microsatellite) using the programme CERVUS (Marshall *et al.* 1998). The output indicated that null alleles were present at microsatellite *Mel116* (+0.2703) which was therefore omitted from the final analysis.

3.8. Trap sample matching

Genetic profiles of vaccinated badgers were matched to the hair trapped population to estimate the percentage of the population that had been vaccinated. Matching was carried out using the statistical package ALLELEMATCH (Galpern *et al.* 2012), executed in R 3.0.2 (R Development Core Team 2016). Samples were matched at all nine of the remaining loci (*Mel116* having been dropped from the analysis; see section 3.7). Profiles that differed from one another by a single mutation (i.e. matching at 17 of a possible 18 alleles) were assigned to the same individual. Error rates were calculated from the data to account for false positive results (matching samples from different animals) and false negative results (failure to match samples from a single animal). In addition, to account for the fact that natural badger movements may have resulted in hair trapped individuals being unavailable for cage trapping and vaccination, a movement rate was estimated for all setts within dispersal distance of the boundary of the IAA. Estimates of the false positive rate, false negative rate and movement rate were used to adjust the raw number of matches in order to produce final estimates of the percentage of badgers vaccinated. Further details of the methodology (error rates, movement rate and matching process) can

be found in Appendix B. Population size was calculated as the number of individuals vaccinated divided by the final estimate of the percentage of the population vaccinated.

3.9. Population model

The cumulative percentage of badgers vaccinated in the IAA by the end of four years of vaccination was estimated using a population model. The model assumed a fixed rate of vaccination (our estimate of the percentage of badgers trapped and vaccinated in 2015), and a fixed rate of survival (based on published estimates of survival). The result represents the estimated percentage of badgers in the population expected to have received at least one vaccine dose by the end of the 2015 vaccination schedule. Details of the population model can be found in Appendix C.

4. Results

4.1. Summary of samples collected and genotyped

A total of 560 hair traps were placed at 72 main setts. The median number of traps per sett was 8 (range 2-18). At six setts, no hair samples were collected. At the remaining 66 setts, the number of samples collected ranged from 1-40. In total 682 trap-day hair samples were collected and submitted for analysis (18 additional trap-day sample bags were submitted but contained no hair sample). Complete genotypes were returned for 384 samples. One hundred and sixteen of these genotypes were identified as being 'mixed' and were excluded from further analyses. This left a total of 268 useable hair trap profiles (39% of all hair trap samples collected).

Plucked hair samples were obtained from 1118 cage trapped and vaccinated badgers of which 1065 were successfully genotyped (95% of all vaccinated badger samples). Initial exploratory analyses identified forty pairs of cage trapped genotypes which matched at all 10 loci. Based on observed allele frequencies the probability of any two badgers being identical at all 10 loci (P_{ident}) is 7.33 e^{-8} or for siblings (P_{sib}), 7.7e^{-4}. Field data showed that these matched samples were collected in relatively close proximity to one another (mean distance 397m, range 0-950m) and on different dates, consistent with recapture. Based on this evidence it was assumed that badgers matching at 10 loci were recaptured individuals. Duplicate genotypes were therefore excluded from further analyses resulting in a final dataset of 1025 useable vaccinated badger profiles.

To account for the presence of recaptures in our data, a recapture probability of 40/1065 (3.8%) was applied to the total number of vaccine doses deployed (1118) resulting in a final estimate of 1076 vaccinated individuals. This figure includes vaccinated individuals for which genotyping was unsuccessful.

Table 1 Summary of samples collected from hair traps and from trapped and vaccinated badgers. Numbers of genotyped samples refer to samples with complete profiles only.

Number of setts trapped	72
Number of hair traps at setts	560
Total number of hair trap samples	682
Number of hair trap samples successfully genotyped	384
Number of hair trap genotypes identified as mixtures	116
Number of hair trap genotypes identified as single animals	268
Number of vaccine doses deployed	1118
Number of cage trapped and vaccinated badger samples successfully genotyped	1065
Number of paired cage trapped and vaccinated genotypes matching at 10 loci	40
Number of unique genotypes from cage trapped and vaccinated badgers	1025
Adjusted number of individual badgers cage trapped and vaccinated	1076

4.2. Trap sample matching

Trap sample matching was carried out at nine loci (*Mel 116* was identified as a null allele and dropped from the analysis; see section 3.7) based on an exact match for at least 17 of a possible 18 alleles.

Within the hair trap genotype database, 141 unique individuals were identified of which 68 (48%) were found to match genotypes of cage trapped and vaccinated animals. This result represents our uncorrected estimate of the percentage of cage trapped and vaccinated badgers in the population.

The false positive rate was estimated by examining the percentage of cage trapped and vaccinated badger genotypes (excluding those matching at all ten loci; see section 4.1) that matched the genotype of another vaccinated badger. In total 47/1025 cage trapped badger genotypes matched at nine loci. Therefore the false positive rate was 4.6%. Of the 47 matches, 13 related to animals trapped on the same morning, confirming that matches had occurred between different individuals.

There were no instances where multiple hair trapped genotypes matched the same cage trapped genotype (multiple matches would indicate that unique hair trapped genotypes had failed to match one another), suggesting that the false negative rate may be low. However, of the original 1118 cage trapped and vaccinated badger hair samples collected, genotyping was unsuccessful for 51. These individuals could therefore not be matched to a hair trapped individual resulting in an effective false negative rate of 51/1118 (4.6%).

Of the 141 unique hair trap genotypes, 86 (60%) related to samples from setts within 2 km of the IAA boundary where movement of animals into and out of the population was a

consideration. For these setts, the movement rate was estimated at between 0-10%, equivalent to 0-6% at the population level.

Incorporating the estimated false positive error rate, false negative error rate and movement rate, the corrected estimate of the percentage of badgers trapped and vaccinated in the IAA in 2015 was 44-65% (95% confidence interval, mean 55%). We also calculated the percentage of badgers cage trapped and vaccinated at each sett (Appendix D).

The estimated size of the badger population within the IAA in 2015 (calculated as the number of individuals vaccinated divided by the percentage of the population vaccinated) was 1645-2457 badgers (95% confidence interval). This is equivalent to a density of between 5.7 and 8.5 badgers per km^2 for an area the size of the IAA (288 km^2)

4.3. Population model

Population modelling estimated that by the end of a four year vaccination programme between 70-85% of the total population would have received at least one vaccine dose, assuming that the percentage of badgers vaccinated in all years is fixed at 44-65%. The same model was used to estimate the percentage of animals with protective immunity after four years of vaccination dependent on the duration of immunity (DOI) conferred by the vaccine and assuming 100% vaccine efficacy (discussed in section 5). The results are shown in Table 2.

Table 2 Population model output showing expected percentage of badgers with protective immunity after four years of vaccination assuming a fixed rate of vaccination of between 44% (lower estimate) and 65% (upper estimate) per year.

	% of population with protective immunity	
Duration of immunity	Lower estimate	Upper estimated
2 years	60	81
3 years	67	84
4 years	70	85

5. Discussion

The present study provides a robust estimate of the percentage of badgers vaccinated in the IAA based on a large and representative sample of setts. No previous studies have estimated badger trapping efficiency as part of vaccine deployment, and there are few published estimates for trapping efficiency during control operations (Smith and Cheeseman 2007). Estimates of the percentage of badgers trapped and vaccinated within the IAA will therefore inform the evaluation of badger TB control strategies both within the IAA and across the wider landscape.

Monitoring of wildlife populations subjected to management is crucial for determining whether the aims and objectives of a given intervention have been achieved. The aim of the present study was to estimate the size of the badger population in the IAA and

subsequently the percentage of that population which had been vaccinated in 2015. As badgers are a nocturnal, fossorial species, producing accurate estimates of population sizes can be difficult (Wilson *et al.* 2003). However, in recent years the development of methods for the remote collection and genotyping of hairs has provided improved opportunities to estimate population size (Frantz *et al.* 2004, Scheppers *et al.* 2007). In the present study we estimated the percentage of badgers that were cage trapped and vaccinated using a genetic approach (trap sample matching) which had previously been applied to estimate culling efficiency (AHVLA 2014).

From the results of the present study we estimate that 44-65% (95% confidence interval) of the resident badger population in the IAA was trapped and vaccinated in 2015. This equates to a population size of 1645-2457 individuals (95% confidence interval) and, for an area the size of the IAA, a population density of between 5.7 and 8.5 badgers per km^2. These results are consistent with the work of Judge *et al*. (submitted) for which the estimated badger density for land class 4 was 4.6-7.4 badgers per km^2 (95% confidence interval). The IAA is largely composed of the broad habitat category 'land class 4' (undulating pastoral land), based on the Land Classification System devised by the Centre for Ecology and Hydrology (Bunce *et al.* 1996).

All methods used for estimating population size rely, to a certain extent, on assumptions. These must be properly understood if the results are to be correctly interpreted. One key assumption in the present study is that the hair trapped badgers were representative of the target population. In order to satisfy this assumption, the setts included in the study were randomly selected, and the sample size was high (approximately 28% of identified main setts within the IAA). Furthermore, hair samples were collected over a 28 day period during which it is likely that most, if not all, individuals would have had been active at the main sett (Scheppers *et al.* 2007) and hence available for trapping. Nevertheless, variation in the duration and intensity of activity at the main sett will influence the probability of an animal being hair trapped and as a result, our hair trapped sample could be biased towards animals that were for whatever reason more active at main setts. It is also possible that our sample is biased towards adult badgers, as cubs may have passed beneath hair traps without coming in contact with the barbed wire. However, as sampling did not take place until June, it will have avoided the main period when very small, post-emergent cubs would be expected to be present.

Another potential source of error in our study is operator bias, which could have been introduced if greater effort was invested in trapping badgers at setts where hair traps had been deployed. This is difficult to assess but if such a phenomenon were widespread then it could have potentially resulted in an overestimate of the true percentage of cage trapped and vaccinated badgers.

Any study involving genotyping is vulnerable to the unwanted effects of genotyping error (mistyping of genotypes due to assay non-specificity, random assay instability or inappropriate allele calling). In the context of the present study, the most likely manifestation of genotyping error is the mismatching of samples from the same individual. We addressed this issue by allowing matches between genotypes that differed by a single

allele. This resulted in a number of matches between different vaccinated individuals, presumed to be false matches. However, this source of error was quantified and incorporated into estimates and should not therefore have biased the result.

The genotyping of DNA from pooled hair samples may have contributed to genotyping error. The approach taken for identifying mixed profiles was rigorous, involving visual inspection of hair trap samples by two operators and the use of parameters specific to both the badger population in the IAA and the microsatellites used for genotyping. The approach was also conservative such that only samples agreed by both operators were included in the analysis. It is still possible that we failed to detect mixtures between two very closely related animals (such that fewer than two alleles occur at all loci), where DNA is contributed approximately equally from both (such that allele peaks are balanced). In this case, the percentage of vaccinated badgers would be under-estimated.

The final estimate of the percentage of badgers vaccinated accounted for the potential movement of 0-10% of animals at all social groups within 2 km of the edge of the IAA. This estimate was based on published rates of badger movement (Appendix B). The incorporation of a movement rate adds uncertainty to the estimate, especially because we used a range rather than a point estimate. If badger movement rates within the IAA (in or out of the area) were significantly higher than this range, then this would result in the estimated percentage of badgers vaccinated being lower. However, higher rates of movement seem unlikely given the apparent high density of the badger population in the IAA, and the tendency for this to be associated with relatively low levels of movement (Rogers *et al.* 1998, Macdonald *et al.* 2008) .

Our population model estimated that by the end of a four year vaccination programme, with a fixed rate of vaccination, 70-85% of the total population would have received at least one vaccine dose. A key parameter in our model was the annual rate of survival for which we used published values from a high density population (Smith *et al.* 2001). If survival rates in the IAA are higher, this would increase the estimated number of badgers vaccinated by year four, as vaccinated badgers would survive for longer. Migration of animals into the population and emigration were not accounted for by the model, but would potentially dilute the vaccinated population. It is therefore possible that the number of badgers which had received one vaccine dose could be higher (due to longer survival) or lower (due to levels of movement) than our estimate of 70-85%.

Although injectable BCG has been demonstrated to be safe and effective in badgers (Lesellier *et al.* 2006, Lesellier *et al.* 2011, Carter *et al.* 2012), it does not induce full protective immunity in all individuals (Chambers *et al.* 2014). This means that some vaccinated badgers may remain unprotected. On the other hand, Carter *et al.* (2012) reported a significant indirect beneficial effect of vaccination in the form of a reduction in the number of seropositive cubs from groups when more than a third of the resident adults had been vaccinated. This beneficial effect is also not captured by our estimate.

In conclusion, the complex epidemiology of bovine TB means that TB control is a challenging task, demanding of a multifaceted approach. As the main wildlife host for TB,

control of infection in badger populations is likely to remain a consideration within the wider landscape of TB control for the foreseeable future. Vaccination has the potential to reduce the incidence of TB in badgers and this may have a beneficial effect on the incidence of infection in cattle. However, a major determinant of the success of any badger vaccination programme is the level of vaccine coverage achieved. The present study represents the first attempt to estimate the level of vaccine coverage accomplished by cage trapping and injecting wild badgers. We have demonstrated that even at the lower estimate of annual coverage, it may be possible to vaccinate in excess of 40% of the badger population, which is consistent with repeated annual vaccination potentially achieving 70% coverage by the end of a four year period.

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.

Bunce, R. G. H., C. J. Barr, R. T. Clarke, D. C. Howard and A. M. J. Lane (1996). "Special Paper: ITE Merlewood Land Classification of Great Britain." Journal of Biogeography **23**(5): 625-634.

Carpenter, P. J., D. A. Dawson, C. Greig, A. Parham, C. L. Cheeseman and T. Burke (2003). "Isolation of 39 polymorphic microsatellite loci and the development of a fluorescently labelled mrker set for the Eurasia badger (Meles meles)." Molecular Ecology notes **3**: 610-615.

Carter, S. P., M. A. Chambers, S. P. Rushton, M. D. Shirley, P. Schuchert, S. Pietravalle, A. Murray, F. Rogers, G. Gettinby, G. C. Smith, R. J. Delahay, R. G. Hewinson and R. A. McDonald (2012). "BCG vaccination reduces risk of tuberculosis infection in vaccinated badgers and unvaccinated badger cubs." PLoS One **7**(12): e49833.

Chambers, M. A., S. P. Carter, G. J. Wilson, G. Jones, E. Brown, R. G. Hewinson and M. Vordermeier (2014). "Vaccination against tuberculosis in badgers and cattle: an overview of the challenges, developments and current research priorities in Great Britain." Veterinary Record **175**(4): 90-96.

Frantz, A. C., F. Fack, C. P. Muller and T. J. Roper (2006). "Faecal DNA typing as a tool for investigating territorial behaviour of badgers (Meles meles)." European Journal of Wildlife Research **52**: 138-141.

Frantz, A. C., M. Schaul, L. C. Pope, F. Fack, L. Schley, C. P. Muller and T. J. Roper (2004). "Estimating population size by genotyping remotely plucked hair: the Eurasian badger." Journal of Applied Ecology **41**: 985-995.

Galpern, P., M. Manseau, P. Hettinga, K. Smith and P. Wilson (2012). "Allelematch: an R package for identifying unique multilocus genotypes where genotyping error and missing data may be present." Molecular Ecology Resources **12**(4): 771-778.

Gill, P., B. Sparkes and J. S. Buckleton (1998). "Interpretation of simple mixtures of when artefacts such as stutters are present - with special reference to multiplex STRs used by the Forensic Science Service." Forensic Science International **95**(3): 213-224.

Hettinga, P. N., A. N. Arnason, M. Manseau, D. Cross, K. Whaley and P. J. Wilson (2012). "Estimating size and trend of the North Interlake woodland caribou population using fecal-DNA and capture–recapture models." The Journal of Wildlife Management **76**(6): 1153-1164.

Judge, J., G. J. Wilson, R. Macarthur and R. J. Delahay How many badgers are there in England and Wales? Estimating badger social group size and abundance from genotyping trapped hairs.

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

Lesellier, S., S. Palmer, D. J. Dalley, D. Davé, L. Johnson, R. G. Hewinson and M. A. Chambers (2006). "The safety and immunogenicity of Bacillus Calmette-Guérin (BCG) vaccine in European badgers (Meles meles)." Veterinary Immunology and Immunopathology **112**(1–2): 24-37.

Lesellier, S., S. Palmer, S. Gowtage-Sequiera, R. Ashford, D. Dalley, D. Davé, U. Weyer, F. J. Salguero, A. Nunez, T. Crawshaw, L. A. L. Corner, R. G. Hewinson and M. A. Chambers (2011). "Protection of Eurasian badgers (Meles meles) from tuberculosis after intra-muscular vaccination with different doses of BCG." Vaccine **29**(21): 3782-3790.

Macdonald, D. W., C. Newman, C. D. Buesching and P. J. Johnson (2008). "Male-biased movement in a high-density population of the Eurasian badger (Meles meles)." Journal of Mammalogy **89**: 1077-1086

Marshall, T. C., J. Slate, L. E. B. Kruuk and J. M. Pemberton (1998). "Statistical confidence for likelihood-based paternity inference in natural populations." Molecular Ecology **7**(5): 639-655.

R Development Core Team (2016). R: A language and environment for statistical computing. Vienna, Austria, R Foundation for Statistical Computing.

Rogers, L. M., R. Delahay, C. L. Cheeseman, S. Langton, G. C. Smith and R. S. Clifton-Hadley (1998). "Movement of badgers (Meles meles) in a high-density population: individual, population and disease effects." Proc Biol Sci **265**(1403): 1269-1276.

Roper, T. (2010). Badger, HarperCollins UK.

Scheppers, T. L. J., A. C. Frantz, M. CSchaul, E. Engel, P. Breyne, L. Schley and T. J. Roper (2007). "Estimating social group size of Eurasian badgers *Meles meles* by genotyping remotely plucked single hairs." Wildlife Biology **13**(2).

Smith, G. C. and C. L. Cheeseman (2007). "Efficacy of trapping during the initial proactive culls in the randomised badger culling trial." Veterinary Record **160**(21): 723-726.

Smith, G. C., C. L. Cheeseman, R. S. Clifton-Hadley and D. Wilkinson (2001). "A model of bovine tuberculosis in the badger Melesmeles: an evaluation of control strategies." Journal of Applied Ecology **38**(3): 509-519.

Wilson, G., S. Harris and G. McLaren (1997). Changes in the British badger population, 1988 to 1997

Wilson, G. J., R. J. Delahay, A. N. S. de Leeuw, P. D. Spyvee and D. Handoll (2003). "Quantification of badger (Meles meles) sett activity as a method of predicting badger numbers." Journal of Zoology **259**(1): 49-56.

Appendix A – Identification of mixed DNA profiles

1. Overview

Identification of mixed genotypes derived from hair trap samples was carried out by visual inspection of the sample electropherogram (the graphical representation of amplified DNA used for determining genotype, EPG). Profiles were classified as mixtures on the basis of:

(i) the presence of more than two alleles at one or more loci and/or

ii) a difference in peak height between heterozygous alleles such that a minimum threshold of heterozygote balance (Hb, see section 3) was exceeded at one or more loci.

The step-wise approach for evaluating hair trap EPGs for evidence of DNA from more than one individual is desrcibed in section 4. Background detail of the methods underlying our approach (which broadly follows the methods of (Gill *et al.* 1998) are described in sections 2 and 3.

2. Stutter

The term ‘stutter’ refers to non-allele peaks which are generated through strand slippage during amplification. Stutter peaks can be one or more repeated length of DNA longer (forward stutter) or shorter (back stutter) than the associated ‘parent’ allele, and at heterozygous alleles, stutter from the larger molecular weight allele can contribute to the peak height of the smaller smaller molecular weight allele (Figure 1). Molecular weight refers to the number of base pairs (bp).

Evaluation against criterion (i) above required prior characterisation of locus-specific stutter observed in single source badger DNA profiles so that stutter artefacts in the hair trap EPGs could be distinguished from true allele peaks.

To establish suitable reference parameters, we sampled, randomly, from the cage trapped and vaccinated badger EPGs in order to calculate locus-specific stutter ratios (defined as the stutter peak height divided by the allele peak height) for up to three back-stutter peaks and one forward stutter peak (Table 1). Homozygous loci were sampled exclusively to ensure that all stutter peaks were attributable to a single allele. Minimum and maximum stutter ratios were used to distinguish between true allele peaks and stutter peaks in the final evaluation of hair trap EPGs (section 4).

3. Heterozygote balance

Heterozygote balance (Hb) is defined as the smallest allele in peak height divided by largest allele in peak height (irrespective of molecular weight). In theory, both alleles at a heterogezygous locus should amplify equally and Hb should be close to 1. In reality, stochastic events, especially when analysing small amounts or poor quality DNA, can lead to allelic imbalances. Differences in allele peak height are therefore not unusual. However, uncharacteristic differences in peak height can indicate the presence of DNA from more

than one contributor. Examples of cases that might present in this way include (1) two homozygous individuals each contributing a different quantity of DNA or (2) a combination of one homozygous individual and one heterozygous individual where one allele is shared by both. In situations where both contributors are very closely related, such that fewer than three alleles are present at all loci, Hb can therefore provide an additional means of identifying mixed DNA profiles.

To establish an appropriate Hb threshold below which profiles should be considered to be of mixed origin, we characterised locus-specific Hb from the cage trapped and vaccinated badger EPGs. The results are shown in Table 2. In cases where the difference in molecular weight between the alleles was 8 base pairs (bp) or less, the peak height of the smaller molecular weight allele was adjusted for the contribution of stutter from the larger molecular weight allele using the median value derived from the reference data (Table 1). In the final evaluation of hair trap EPGs we chose the locus-specific median value ± 3 standard deviations as the threshold value for Hb.

4. Evaluation protocol

Finally, each hair trap EPG was evaluated according to the following steps:

i. On a locus by locus basis, confirm that all peaks, other than those specified as allele peaks, can reasonably be attributed to stutter. Evaluation was informed by both the locus-specific parameters given in Table 1 and the profile of observed peaks relative to adjacent stutter. For example, a peak height falling outside of the minimum and maximum range specified could still be classified as stutter based on its relative height within a sequence of stutter peaks.
ii. On a locus by locus basis, confirm that the balance of peak heights (Hb) (where appropriate) does not exceed a specified threshold value. The threshold value specified in this study was the locus-specific median value for Hb ± 3 standard deviations (Table 2).
iii. Form a final assessment, based on information from all loci as to whether this EPG reflects DNA from more than one individual. Classification was conservative such that any profile that could not confidently be attributed to a single individual at all ten loci was assumed to be mixed and, as a result, excluded from further analysis.

This process was carried out by two independent operators. Only profiles assessed by both operators as being of single source DNA were included in the trap sample matching analysis.

Table 1 Stutter ratios (stutter peak height divided by parent allele peak height) for back stutter (up to 3 peaks of decreasing height and decreasing number of base pairs) and forward stutter (one peak only) associated with alleles at each of 10 microsatellite markers. Data were derived from randomly selected homozygous loci (n=20) from the cage trapped and vaccinated badger database.

	first back stutter (%)				second back stutter (%)				third back stutter (%)				forward stutter (%)			
locus	range	mean	median	std	range	mean	median	std	range	mean	median	std	range	mean	median	std
Mel103	45-80	58	58	7	11-27	20	20	3	3-8	6	6	1	4-7	5	5	1
Mel104	50-73	63	65	6	13-37	23	23	6	5-15	9	9	3	3-15	6	5	3
Mel105	25-75	57	58	10	10-37	22	21	6	1-15	8	8	3	5-12	8	8	2
Mel107	45-69	54	54	6	16-30	21	20	4	7-12	8	8	2	2-18	8	8	3
Mel110	73-98	82	82	8	30-55	39	37	8	10-26	16	15	4	5-18	8	8	3
Mel113	46-82	60	59	9	13-25	18	18	4	3-9	5	5	2	1-4	3	3	1
Mel114	39-63	50	48	6	11-21	15	15	2	3-6	4	4	1	3-5	4	4	1
Mel115	1-13	9	9	2	1-1	1	1	0	1-1	1	1	NA	1-5	2	2	1
Mel116	30-65	41	40	9	6-25	11	11	5	1-9	3	3	2	2-23	9	9	6
Mel117*	8-38	19	14	11	1-33	6	1	10	2-6	4	4	3	1-4	2	1	1

*** There were only 10 individuals that were homozygous at this locus. Therefore the sample size for this locus was 10 and not 20 as for the other nine loci.**

Table 2 Heterozygote balance (smallest allele in peak height / largest allele in peak height (irrespective of molecular weight)) at all heterozygous loci in the cage trapped and vaccinated badger database.

	heterozygote balance (%)			
locus	range	mean	median	std
Mel103	16-100	83	84	11
Mel104	21-100	75	75	12
Mel105	27-100	85	86	10
Mel107	44-100	86	87	10
Mel110	15-100	76	78	17
Mel113	7-100	76	77	15
Mel114	30-100	87	90	11
Mel115	19-100	84	86	12
Mel116	7-100	59	57	22
Mel117	14-100	81	88	19

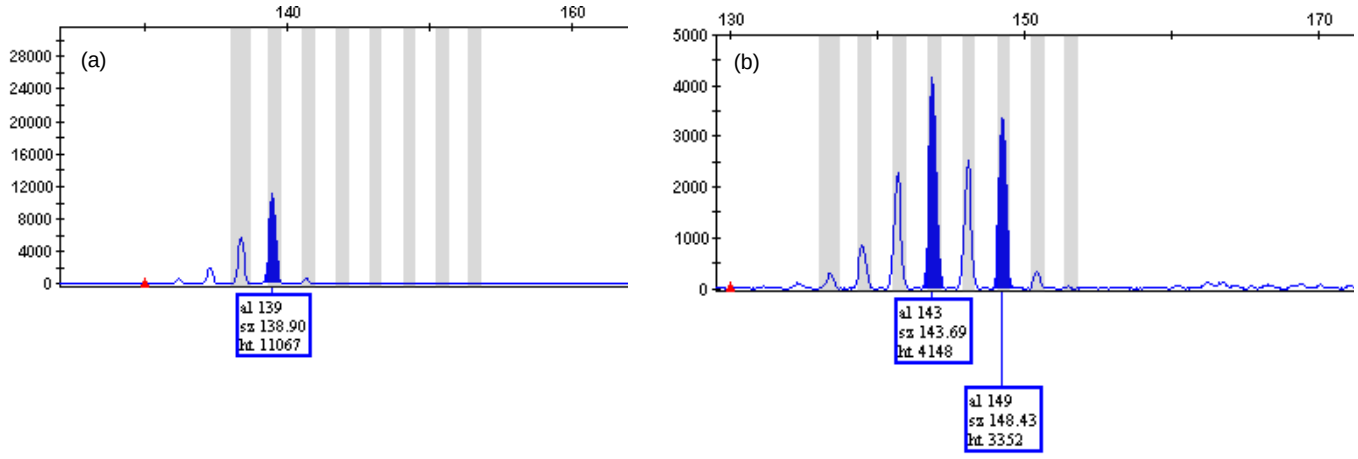

Figure 1 (a) Screen grab of a dinucleotide microsatellite EPG for a homozygous individual where the true allele peak (solid blue) is associated with three visible back stutter peaks (outline only) and a single forward stutter peak and (b) Screen grab of a dinucleotide microsatellite EPG for a heterozygous individual where each of the true allele peaks (solid blue) have associated stutter peaks and where stutter from the larger allele (149 bp) has contributed to the height of the second allele peak (143 bp).

Appendix B – Details of trap sample matching

1. Principles of the method

The trap sample matching methodology reported here is similar to the 'cull sample matching' methodology used to evaluate the pilot culls in Gloucestershire and Somerset (AHVLA 2014). Trap sample matching involves the genotyping of remotely sampled hairs to create a target population of genetically unique individuals which are present within the population. This target population is then compared to the genotypes of vaccinated (cage-trapped) individuals to identify matches indicating which of the known badgers in the population were trapped and vaccinated. The percentage of hair trapped individuals which match cage trapped individuals gives an estimate of the percentage badgers vaccinated in the population.

2. Identifying unique individuals and genetic matches

Each individual animal, with the exception of identical twins, has a unique genetic code. Genetic profiling techniques aim to characterise enough of this code such that individual animals can be accurately identified. We sequenced hair samples at 10 genetic markers, with one of these (Mel116) excluded from the analysis due to the high presence of null alleles (section 3.7). Every marker yields two alleles (one from each parent), such that the genetic profile of each sample consists of 18 alleles (9 markers X 2). Only hair samples with complete genetic profiles (where DNA amplified at all nine markers) were included in the analyses.

Genetic matches between hair samples were identified using the statistical package *ALLELEMATCH* (Galpern et al. 2012) using program R. *ALLELEMATCH* starts with pairwise comparisons between samples, estimates a similarity score between each pair of profiles, and then uses clustering to find groupings of similar profiles that likely belong to a single individual. *ALLELEMATCH* is particularly well suited to studies such as this one where genetic samples are obtained by remote sampling (e.g. hair traps) resulting in variable sample quality and potential for genotyping errors (Galpern *et al.* 2012). Genotyping errors ('stutter' or allelic dropout) may mean that genetic profiles differ slightly despite being obtained from the same individual. In the current study samples were identified as being from the same individual if they matched at 18 alleles (all alleles/loci) or 17 alleles (i.e. they differed at one allele). The decision was taken to match individuals if they were genetically identical at ≥17 alleles in order to minimise the likelihood that genotyping errors would result in failures to match, or the creation of new false genotypes. Matching samples that differ at one, or a small number of alleles is commonly used in similar wildlife genetics studies (Hettinga *et al.* 2012). *ALLELEMATCH* calculates P_{sib} (the probability of the observed match occurring between siblings based on observed allele frequencies) for each genetic match and unique genotype. A threshold P_{sib} value can also be used to remove matches which may have occurred by chance (Galpern *et al.* 2012), with a cutoff of <0.05 commonly used (Hettinga *et al.* 2012). All matches and unique

genotypes in the current study had P_{sib} values of <0.05 (mean=0.0017, min=0.0002, max=0.006).

3. False negatives (failures to match)

A false negative result can occur if two genetic samples fail to match despite coming from the same individual. Failures to match could occur due to genotyping errors at >1 alleles. Frantz *et al.* (2006) did not identify any obvious genotyping errors from 749 badger hair samples, so it was assumed that false negative rates would be low. In the current study, false negative results would inflate the number of unique hair genotypes collected at each sett which could result in an underestimate of the percentage badgers vaccinated. Previous work using hair samples to estimate culling efficiency estimated false negative rates by comparing the rate that distinct hair trapped genotypes matched the same culled badger (ear tip) but not each other (AHVLA 2014). In such instances it was assumed that the two hair genotypes were from the same individual but had failed to match. The effect of these 'missed matches' on the percentage badgers vaccinated is estimated from the binomial proportion of the number of unique vaccinated genotypes matching hair trapped genotypes against the number of unique hair trap genotypes matching vaccinated genotypes (AHVLA 2014). For example if 10 vaccinated genotypes matched 12 unique hair genotypes this would result in a false positive rate of 1- 10/12 (9%).

Another potential source of false negative result is the fact that full usable genetic profiles were not obtained from all vaccinated badgers. If a genetic profile is not generated for each vaccinated badger, there is a possibility that a badger is hair trapped and subsequently cage trapped but no sample is available to be matched. This source of error is estimated from the binomial proportion of the number of cage trapped badgers with useable genetic profiles divided by the total number of vaccinated badgers sampled.

4. False positives (mismatches)

False positive results, where two samples are incorrectly matched to one another could occur could occur due to genotyping errors producing erroneous matches, or because closely related individuals were genetically identical at ≥17 alleles. False positives could result in overestimates of the percentage badgers vaccinated by artificially inflating the number of matches between hair trapped genotypes and vaccinated genotypes. As a consequence, all estimates of percentage badgers vaccinated were corrected for an estimated mismatch rate.

The expected number of mismatches between vaccinated badgers and hair trapped badgers was estimated from the number of matches among vaccinated badgers which are assumed to all unique individuals. If 10% of vaccinated genotypes match other vaccinated genotypes, this indicates that there is a 10% chance (± error) of falsely matching hair trapped genotypes to vaccinated genotypes. For example, if the number of hair trapped genotypes matching vaccinated genotypes was 10/20 (50%) with a 10% mismatch rate, this would result in an adjusted rate of 9/20 (45%).

5. Estimating the movement rate

The assumption underlying trap sample matching is that hair trapped individuals are available to be cage trapped. However, the hair trapping and cage trapping was not carried out simultaneously at all setts. As a consequence, it is possible that hair trapped individuals are not available to be caught, either because they moved out of the area before they could be vaccinated (if vaccination occurred after hair trapping) or because they moved into the area after vaccination occurred (if hair trapping occurred after vaccination). It is not possible to determine exact movement rates at each badger sett during this project. Long-term monitoring of badger populations suggests that movement occurs between badgers social groups in ~10% of trapping events (Rogers *et al.* 1998), with individuals moving on average 0.4-1km with most movements <2km. The rate of movement in the current study was estimated as 0-10% at social groups within 2 km of the outer boundary of the IAA.

6. Estimating the percentage of badgers vaccinated

The percentage of badgers vaccinated was estimated as the percentage of hair trapped genotypes matching vaccinated genotypes with correction for false positive match rate, false negative match rate and movement rate. This was implemented using a second order Monte Carlo estimate with the following steps:

1. Estimate a distribution for the false negative probability from missed matches among hair trap genotypes (FN_1).
2. Estimate a distribution for the false negative probability from failures to genotype vaccinated badgers (FN_2).
3. Estimate a distribution for the false positive rate from matches among vaccinated badgers (FP).
4. Estimate a distribution for the probability of movement (FE).
5. Select independent random quantiles from each of the distributions
6. The percentage vaccinated badgers is the number of returned badgers (matches with vaccinated genotypes, x) divided by the number of hair trapped individuals n, expressed as a percentage $\frac{x}{n} \times 100$.
7. The number of returned badgers, x, is adjusted downwards by the effective false positive rate eFP (random binomial draw with probability, FP with size $.x$)
8. The number of hair trapped individuals *n* is adjusted down by the effective false negative rates eFN_1 (random binomial draw with probability, FN_1 and size=n), eFN_2 (random binomial draw with probability, $FN2_1$ and size= n) and also by the effective population movement eFE (random binomial draw with probability = FE and size=n).
9. The adjusted percentage vaccinated is calculated by (x- eFP) / (n - eFE - eFN_1 eFN_2)
10. The above steps are carried out for each individual sett and for the whole population 1000 times.
11. The mean estimate for each sett is the mean across each 1000 repeats, while the 95% confidence intervals are the 2.5th and 97.5th percentiles of the distribution.

12. On each of the 1000 repeats the population level estimate is a random quartile from the binomial proportion (x - eFP) / (n - eFE - eFN_1 - eFN_2). The 95% confidence intervals are the 2.5^{th} and 97.5^{th} percentiles of this distribution.

Appendix C - Population model

1. Model framework

A population model was created to estimate the cumulative percentage of vaccinated badgers over a four year vaccination programme. The model consisted of a series of equations outlined below.

The badger population P is composed of a total number of adult males M, adult females F and cubs C.

$$P = M + F + C$$

In a given year, t, the total number of badgers vaccinated is V_t, comprised of vaccinated adult males Vm_t, vaccinated females Vf_t and vaccinated cubs Vc_t.

$$V_t = Vm_t + Vf_t + Vc_t$$

Where Vm_t, Vf_t and Vc_t are given by:

$$Vm_t = M \times R$$

$$Vf_t = F \times R$$

$$Vc_t = C \times R$$

Where R is a constant value specified from the estimate of percentage badgers vaccinated (i.e. 44-65%).

At the start of year one of the programme, there will be no previously vaccinated badgers. At the start of years two, three and four, the adult male and adult female components of the population may contain surviving badgers vaccinated in previous years.

$$M_t = Sm_t + Um_t$$

$$F_t = Sf_t + Uf_t$$

Where M_t is the adult male badger population and F_t is the adult female badger population. Parameters Um_t and Uf_t are equal to the number of unvaccinated adult male and adult female badgers respectively. Parameters Sm_t and Sf_t are equal to the number of male and female vaccinated badgers surviving from previous years. Sm_t and Sf_t are determined by the annual survival rate of adult badgers and also the survival rate of badger cubs, which become adults in the following year. In year two the number of surviving adult vaccinated badgers will therefore depend on the surviving vaccinated adults and cubs from year one

$$Sm_2 = (Vm_1 \times Qm) + 0.5(Vc_1 \times Qc)$$

$$Sf_2 = (Vf_1 \times Qf) + 0.5(Vc_1 \times Qc)$$

Where Qm, Qf, and Qc are the survival rates for adult males, adult females and cubs respectively. Badger cubs have approximately a 50:50 sex ratio (Roper 2010) hence the 0.5 in the equations above.

In year three the number of surviving vaccinated adult badgers will depend on the number of vaccinated adults and cubs from year two which have survived (which will contain some animals vaccinated in year one and two), along with surviving adults which were vaccinated in year one only (which have survived for two years).

$$Sm_3 = (Vm_2 \times Qm) + 0.5(Vc_2 \times Qc) + (Sm_2 \times Qm \times 1 - R)$$

$$Sf_3 = (Vf_2 \times Qf) + 0.5(Vc_2 \times Qc) + (Sf_2 \times Qf \times 1 - R)$$

In year four the number of surviving vaccinated adult badgers will depend on the number of vaccinated adults from year three, year two and year one.

$$Sm_4 = (Vm_3 \times Qm) + 0.5(Vc_3 \times Qc) + (Sm_3 \times Qm \times 1 - R)$$

$$Sf_4 = (Vf_3 \times Qf) + 0.5(Vc_3 \times Qc) + (Sf_3 \times Qf \times 1 - R)$$

Therefore in year t the total number of badgers in the population which have received at least one vaccine dose is the number of badgers vaccinated in that year (some of which will have been vaccinated previously) plus surviving animals vaccinated in previous years which were not vaccinated in year t.

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

Equations were solved using the parameters in Table 1 (next page).

Table 1 Parameters used for the population model. 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
V	% badgers vaccinated	44-66%
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

The size of the adult male population M, adult female population F and cub population C are also displayed in table 1. These numbers sum to 100 and are based on the percentage of the badger population which are composed of these three different components. For example, parameter C is 32.45 or 32%, indicating that roughly 1/3 of a badger population is composed of badger cubs. These values are fixed for the purposes of the model, such that neither the size nor the demographic of the population fluctuates over time. The model does not include a specific parameter for emigration or immigration. Movement of vaccinated animals out of a population, or the movement of unvaccinated into the population will result in a reduction in the vaccinated percentage of the population. As a result, our model potentially overestimates the percentage of the population vaccinated. However, survival parameters Qm, Qf, and Qc are based on a high density population of badgers. If the rate of survival in the IAA is higher than the parameters used here, this could result in the actual percentage vaccinated exceeding our modelled estimate. A lower survival rate would result in the actual percentage vaccinated being lower than our modelled estimate.

2. Varying the duration of immunity (DOI)

Our model was initially used to estimate the total percentage of badgers receiving at least one vaccine dose after a four year vaccination programme. The duration of immunity (DOI) in badgers vaccinated with injectable BCG is not known. If the DOI is four years, then all badgers receiving at least one vaccine during a four year programme will be protected against infection by year four.

To explore the effect of DOI on overall population protection we varied the model to account for a potential DOI of two years and three years.

Varying the DOI influences the number of surviving protected adult female and adult male badgers in a given year t, defined by parameters Sm_t and Sf_t. .If the DOI is one year, the number of vaccinated animals is simply determined by the parameter R (percentage badgers vaccinated) and parameters Sm_t and Sf_t (surviving adult badgers vaccinated in previous years) are equal to zero If the DOI is 2 years the number of surviving protected badgers in given year t only includes adult badgers and cubs vaccinated in the previous year.

$$Sm_t = (Vm_{t-1} \times Qm) + 0.5(Vc_{t-1} \times Qc)$$

$$Sf_t = (Vf_{t-1} \times Qf) + 0.5(Vc_{t-1} \times Qc)$$

It the DOI is 3 years the number of surviving protected badgers in year t includes surviving vaccinated adult badgers and cubs from the previous year t-1, along with surviving adults vaccinated in year t-2 only.

$$Sm_t = (Vm_{t-1} \times Qm) + 0.5(Vc_{t-1} \times Qc) + (Vm_{t-2} \times Qm^2 \times 1 - R) + 0.5(Vc_{t-2} \times Qc \times Qm \times 1 - R)$$

$$Sf_t = (Vf_{t-1} \times Qf) + 0.5(Vc_{t-1} \times Qc) + (Vf_{t-2} \times Qf^2 \times 1 - R) + 0.5(Vc_{t-2} \times Qc \times Qf \times 1 - R)$$

Appendix D - Sett-level variation

Our main estimate of the percentage of cage trapped and vaccinated badgers (44- 65%) was calculated at the population level (i.e. total matches from all setts/total hair trapped population). We also calculated estimates and mean values for the percentage of badgers vaccinated at each sett. The difference between the population estimate and the sett level estimate and the difference in uncertainty associated with each estimate provides an indication of how between-sett variation may have impacted the population estimate.

Sett-level estimates for the percentage of badgers trapped and vaccinated from each social group varied between 0-100 with a mean value of 51% (40-61% 95% confidence interval). There were limitations associated with these data, primarily due to low sample size at a number of setts. None the less, the sett-level estimate compares favourably with population level estimate (44-65% 95% confidence interval, mean 55). This suggests that sett level variation in the number of genetic matches (Table 1, Figure 1) did not significantly influence the overall population estimate.

Table 1 Summary of genotyped hair trap samples which matched vaccinated badger genotypes at each sett.

Sett ID	No. hair trap samples genotyped	No. hair trapped individuals	No. matching vaccinated individuals	% matched*
1999	2	1	0	0
591	2	1	0	0
4547	1	1	1	100
6293	1	1	1	100
4726	1	1	0	0
4173	3	1	1	100
316	1	1	0	0
1669	1	1	0	0
5827	1	1	1	100
3098	1	1	0	0
1092	2	1	0	0
7713	1	1	1	100
5500	1	1	0	0
2518	5	1	1	100
1452	1	1	1	100
6981	1	1	1	100
6837	1	1	0	0
4415	1	1	1	100
1773	1	1	1	100
6701	1	1	1	100
6713	1	1	1	100
91	1	1	0	0
496	2	2	2	100

Sett ID	No. hair trap samples genotyped	No. hair trapped individuals	No. matching vaccinated individuals	% matched*
1618	6	2	1	50
8725	4	2	2	100
5638	4	2	1	50
4667	11	2	0	0
3379	2	2	1	50
7002	2	2	1	50
1326	3	2	1	50
4034	4	2	0	0
209	2	2	0	0
1431	4	3	2	67
270	9	3	1	33
8032	5	3	1	33
63	4	3	0	0
29	5	3	1	33
205	5	3	0	0
6307	9	4	2	50
8210	8	4	1	25
4638	7	4	2	50
5026	11	4	3	75
6557	13	4	3	75
4609	6	5	1	20
5118	7	5	1	20
2695	6	5	2	40
4153	9	5	3	60
2653	13	6	6	100
5860	8	6	4	67
4323	8	6	0	0
2364	18	7	4	57
2458	17	9	6	67
5859	25	10	5	50

*** '% matched' in this table is the percentage of hair trapped individuals present at a sett which match vaccinated individuals. These are the raw numbers which have not been adjusted for false positive, false negative and movement rates.**

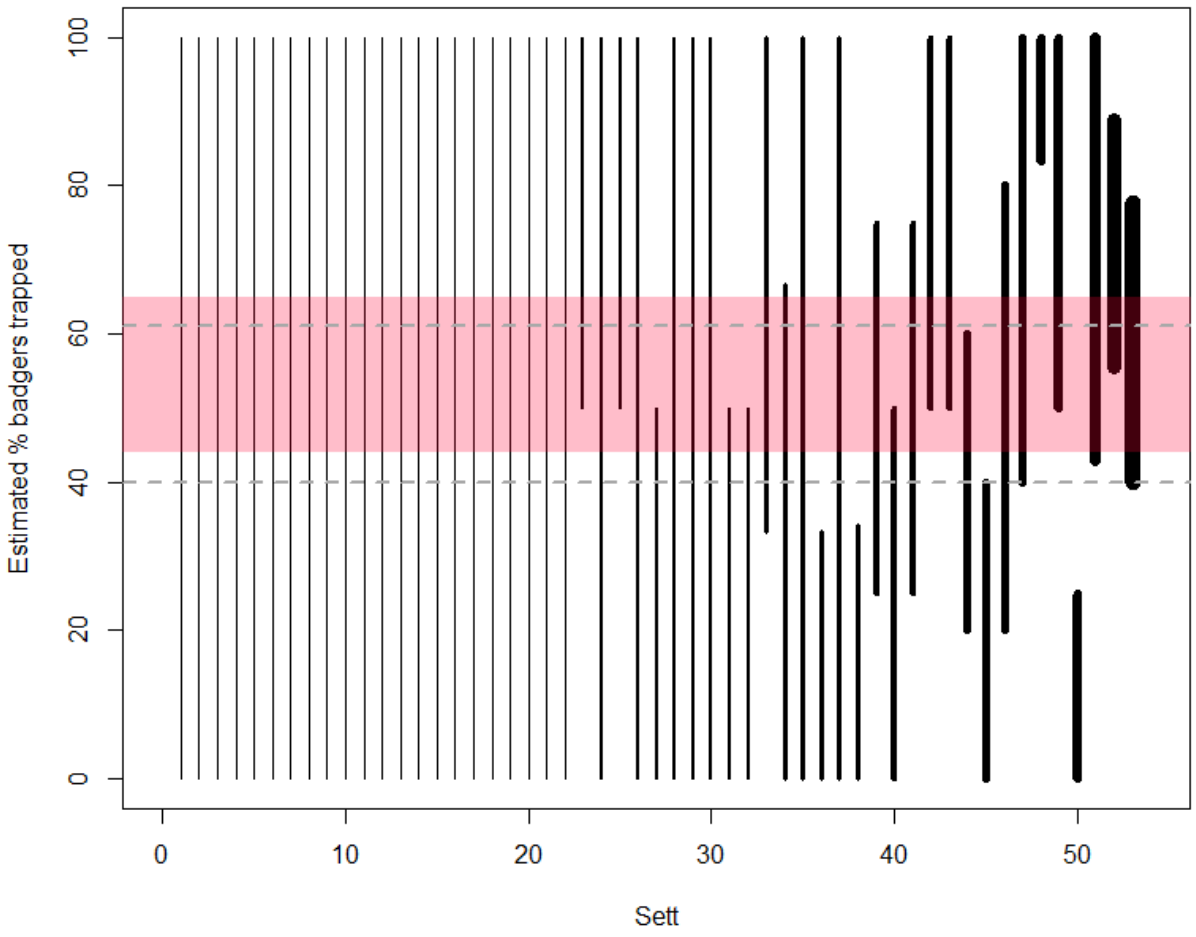

Figure 1 Sett level estimates of the percentage of badgers trapped and vaccinated in the IAA in 2015. The percentage estimates are represented by vertical lines in black. The width of each line is proportional the number of hair trapped and genotyped badgers at each sett. The pink horizontal band shows the 95% confidence limit for the population-level estimate of the percentage of the population trapped and vaccinated and the dotted grey lines indicate the 95% confidence limits of the mean sett-level estimate.