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RESEARCH

Performance of fur clips and livestock markers for identifying vaccinated badgers

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Abstract

Marking free-living wild mammals may be desirable during field studies and management interventions; however, doing so presents practical challenges. In the context of disease management interventions, different approaches such as vaccination and culling may be deployed in adjacent areas resulting in a need to identify previously vaccinated individuals to avoid losses of vaccination benefits. Badgers (*Meles meles*) have been identified as a wildlife reservoir of bovine tuberculosis in several countries. In England, the primary means of controlling disease in badger populations has been culling, although policy also includes the use of badger vaccination. Vaccination and culling can therefore increasingly take place in adjacent areas. The current means of marking vaccinated badgers is to apply a fur clip; however, the performance of this method has not been assessed. In this study, we assessed the field performance of livestock markers not previously trialled on badgers. We also assessed the performance of fur clips in terms of (a) how likely they are to be detected on recapture and (b) their detectability using remote cameras. None of the livestock markers trialled persisted well on badger fur. Detectability of fur clips on re-captured badgers fell to 50% in adult badgers by approximately 3 months from application. In cubs, detectability fell to less than 50% within 3 weeks of application. We suggest it is highly likely that, if vaccination and culling were carried out in adjacent areas and fur clipping was the primary means of determining vaccination status, a proportion of recently vaccinated badgers would be removed, particularly cubs. This has important implications for disease control, and we suggest options for minimising such losses.

Keywords Marking · Recapture · Badgers · Vaccination · Bovine tuberculosis

Introduction

Marking free-living wild mammals for future identification can be a key challenge for field studies and management (Powell and Proulx 2003). Frameworks have been established to guide researchers in choosing the most appropriate methods of marking given the required duration of the mark, the welfare costs of trapping or restraining the animal to administer it, and the direct welfare impact of the mark itself (Lane and McDonald 2010; Silvey 2020). A wide variety of approaches have been reported in the literature (see Silvy et al. (2012) for a detailed review of marking methods for wildlife) including external ringing and tagging (e.g. bird rings, mammal ear tags), physical marking (e.g. tattooing, fur clipping, or applying paints/dyes), internal marking (e.g. micro-chipping), and the use of variable natural markings (e.g. identifying individually distinct patterns or coloration, most recently using machine learning technologies (Petso et al. 2021)).

Individual identification of wildlife may be desirable during disease management interventions, for example to identify previously vaccinated animals, assess vaccine coverage in a population, and plan intervention strategies (Childs et al. 1998). Biomarkers have previously been used to identify individual animals that have consumed vaccine baits (Pedersen et al. 2018; Robertson et al. 2022). However, to ascertain the presence or absence of the biomarker, biological samples (blood or hair) must be collected and analysed, so this approach would not permit an individual to be identified in real-time by observation under field conditions. Instead, rapid identification of individuals in the field is likely to require the application of a visible mark of some kind.

Vaccination is currently deployed for the control of disease in wildlife (most notably rabies and bovine tuberculosis)

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and is under development for many others (Cross et al. 2007; Barnett and Civitello 2020). In the case of bovine TB, a global livestock disease caused by infection with *Mycobacterium bovis*, wildlife vaccination is in use or under consideration as a disease control strategy in several countries (see review in Buddle et al. (2018)) including South Africa (Arnot and Michel 2020), New Zealand (Aldwell et al. 2003), the USA (Palmer et al. 2007), Spain (Gortazar et al. 2014), Republic of Ireland (Martin et al. 2020), and the UK (Benton et al. 2020, Smith et al. 2020).

In England, the costs of dealing with bovine tuberculosis in the national cattle herd exceed £100 million annually (Defra 2021). Although many species of wild mammal can be infected with *M. bovis* (Delahay et al. 2005, Delahay et al. 2007), the European badger (*Meles meles*) is considered to be the primary wildlife reservoir of infection and transmission between badgers and cattle has been repeatedly demonstrated (Crispell et al. 2019, van Tonder et al. 2021; Rossi et al. 2022). Management of TB infection in badger populations in England has largely focused on badger culling, undertaken by government staff (Krebs et al. 1997) and more recently by the farming industry (by cage trapping and dispatch or shooting (Downs et al. 2019)). However, badger vaccination (achieved by cage-trapping and intra-muscular injection with the licensed live vaccine; BadgerBCG) has been available as a disease control tool since 2010 following the demonstration that it could reduce the severity of disease progression in badgers (Chambers et al. 2011). Although field deployment of the vaccine has been relatively limited in the past (Benton et al. 2020), it has recently been the subject of renewed focus (Defra 2020a, b). Where vaccination and culling are taking place in adjacent areas and where the decision to remove an individual animal would ideally depend on its prior vaccination status (to avoid losing the benefits of vaccination), it would be desirable to be able to easily determine vaccination status in real time under field conditions. Hence, there is interest in exploring means of reducing the likelihood that recently vaccinated badgers are removed during culling. This challenge is complicated by the absence of definitive information on the duration of the protective effect of vaccination in badgers (Carter et al. 2012). Studies of BCG performance in vaccinated calves note a significant protective effect at 12 months old which is however undetectable by 24 months (Thom et al. 2012). If it were the case that BCG vaccine performs similarly in badgers, then the duration of the protective effect might be estimated at 12–18 months. We have no reason to expect that vaccination with a single dose of BCG provides longer or even lifelong protection. For this reason, ideally a badger would need to be readily identifiable for at least 12 months after vaccination.

Currently, vaccinated badgers are fur clipped on the back or rump, and the exposed underfur is sprayed with livestock marker whilst they are fully conscious in the trap (Natural England 2023). This mark is primarily intended to allow the animal to be identified on a second night of trapping so that it can be released without being unnecessarily vaccinated more than once (the vaccine is licenced on the basis of annual vaccination). The standard livestock marker spray used for this purpose usually wears off within a few days. Other physical markers have been used on badgers, for example Nyanzol D dye (Fitzwater 1943; Stewart and Macdonald 1997) which was found to perform well on badger fur, although serious concerns have since been raised as to its toxicity (Milman 2019). More invasive approaches have been used to mark badgers including ear tags and tattooing of the abdomen (Cheeseman and Harris 1982), although both require anaesthesia. Subcutaneous microchipping has also been carried out under anaesthesia (Lesellier et al. 2006; Woodroffe et al. 2017; Aznar et al. 2018; Ham et al. 2019; Menzies et al. 2021) and in limited circumstances on conscious badgers during vaccination operations (although in the UK this can only be performed by registered veterinary surgeons (RCVS, *pers comms*)), and uncertainty remains as to whether microchips placed in conscious badgers are consistently readable on recapture. Genetic markers have been used to distinguish individual badgers on the basis of the unique microsatellite marker patterns derived from hair samples. Hair can be collected without anaesthesia using hair traps deployed in the field or directly from trapped badgers (Smith et al. 2020), although because samples require processing in the laboratory, this approach is not suitable for trap-side identification of previously vaccinated badgers.

The use of visible marks such as fur clips, paints, or dyes is preferable to more invasive methods (e.g. microchipping or tattooing) as they are low-cost, easier to apply, and likely to have a lower welfare impact on the marked individual (Haines et al. 2018). In the present study, we trialled a range of candidate coloured markers, assessing their suitability for application to a conscious badger under field conditions without the need for extended restraint or anaesthesia. It was acknowledged at the outset that the likely duration of any fur clip or mark applied to the pelage of badgers would be constrained by their annual moult cycle. In adults, this typically starts in June/July ending in Nov/Dec. Yearling badgers (those in their second year) commence and complete moulting earlier (May/June–Oct/Nov), whilst cubs exhibit continuous hair growth and do not moult (Roper 2010). We assessed the detectability of candidate markers alongside the standard marking approach (fur clipping the guard hairs of the badger through the bars of the cage-trap (Natural England 2023)) by operators working under field conditions and by remote camera surveillance. We also explored variation in the longevity of visible marks in relation to badger age and the severity of the mark itself.

Materials and methods

Selection of potential markers

A literature search was conducted on the Scopus database of peer-reviewed literature for journal articles published using the search terms 'dye AND wildlife' and 'marking AND wildlife'. No date range was set, and articles returned covered the period 1972–2022 yielding over 400 results. Article titles and abstracts were screened for potential relevance to the required application, reducing the number to 208 results. Professional networks (colleagues within the APHA National Wildlife Management Centre team, university contacts, correspondence with those who had published work previously using potentially relevant marking approaches) were also used to seek information on potentially relevant approaches from other wildlife professionals. We also explored novel options from other industries with potential value, for example anti-theft marker sprays. As we required a marker that could be applied under field conditions, without anaesthesia by non-veterinary personnel, we focused on identifying potentially suitable dyes, inks, or sprays. Some products used to mark wildlife such as hair dye and hydrogen peroxide (White et al. 1980, Schooley et al. 1993) carried an unacceptable risk of adverse welfare impacts and/or were impractical to apply under field conditions; hence, we further restricted our search to products which had already been approved for use in livestock. Once a panel of candidate markers had been selected, consultation on their use in field trials took place with the APHA Animal Welfare and Ethical Review Board (AWERB) and the UK regulatory authority. Subsequently, an approved short list of proposed marking approaches deemed to be at low risk of causing adverse effects and suitable to take forward to field trials was identified as follows:

1. Marking paste developed for use in sheep.
2. Branding fluid used for long-lasting identification of sheep and lambs.
3. Oil-based livestock tail paint crayon developed for marking cattle.

Field trials

Field studies took place at Woodchester Park, Gloucestershire between July 2020 and November 2021. Badger setts (underground burrow systems) in the study area have been routinely trapped, up to four times a year, since 1976. The full procedure has been described elsewhere (McDonald et al. 2018), but, in brief, following capture, badgers are transported to a sampling facility where they are anaesthetised and sampled before being returned to their sett following recovery. Before testing on live badgers, the selected livestock markers were applied to a badger pelt as an initial indication of their likely visibility on a live badger. Failure to produce a clear mark at this point would result in the approach not being progressed to field trial. In year 1, the performance of the above panel of livestock markers was tested, and in year 2, the performance of fur clipping alone was assessed.

Assessing the detection of livestock markers by field operatives

The original intention of the study was to apply marks to badgers during the spring and summer (May/June), representing the likely start of any vaccination campaign, and then to assess their longevity through to the end of November when the vaccination trapping season ends (Natural England 2023)). However, COVID-19 restrictions severely limited fieldwork activities in 2020, and so marking badgers was delayed until midway through the summer trapping season (July 2020). Consequently, markers were not assessed over the entire period of interest, and so year 1 work provided only limited evidence on their persistence. During routine trapping and sampling operations in the study area, badgers were marked in the field after they had been sampled but immediately prior to release back to their sett. This was primarily to avoid any wet marks applied to a badger rubbing off during routine sampling and also replicated the situation during a vaccination operation whereby they would be released immediately after marking. Hence, each badger was taken back to its sett for release as usual where it was run back into a cage trap (as if it had just been captured) and fur clipped using curved scissors inserted through the bars of the trap (5 × 5 cm mesh) and marked. The use of battery-operated pet clippers was also trialled as an alternative to scissors for applying a fur clip. Trappers were instructed to apply clear fur clips on the back or rump of badgers, aiming for a mark about the size of a playing card (approximately 9 × 6 cm). It was expected that in this scenario, the badgers being marked were likely to be more agitated than under normal vaccination trapping conditions when the animal would have been in a trap for several hours. Hence, application of the mark under the study conditions represented a worst-case scenario test. However, owing to the more animated behaviour of the trapped badgers in the field, a decision was taken to also apply marks to a sample of anaesthetised badgers to assess whether the mark might persist if it could be applied in a more controlled manner. Photos were taken of all marks applied in the field and under anaesthesia.

Box 1 Examples of fresh fur clips applied under field conditions on a conscious badger (using curved scissors through the bars of a cage trap), graded by size and depth. Photos taken whilst captured badgers were anaesthetised during sampling, which took place immediately after fur clips had been applied in the field.

Assessing the detection of fur clips by field operatives

In the second year of the study (2021), fur clips alone were applied under field conditions only to badgers at their first capture of the year (after capture but prior to subsequent anaesthesia and sampling). Subsequent routine trapping events in 2021 provided an opportunity to assess whether marks were visible under field conditions. Photos of fur clips were taken when freshly applied and at all subsequent re-capture events. As all badgers captured during the Woodchester Park study are permanently tattooed when first caught, this provided a robust means of identifying fur-clipped individuals. After field application, once the animal was under anaesthesia, the size of each fur clip was graded relative to the size of the individual animal rather than a prescribed measurement, mainly over the rump area which is where most of the fur clips were applied. A 'small' fur clip would cover an area estimated to be less than 20% of the width of the badger; a 'medium' clip would cover between 20 and 40%, and anything larger would be classed as a 'large' clip. Fur clips were also graded by depth ('shallow': only the dark guard hairs trimmed, 'medium': light undercoat visible and some undercoat clipped, and 'deep': undercoat clearly clipped) (see Box 1). All gradings of size and depth were carried out by the same researcher.

To test for variation in detectability of fur clips under field conditions, we constructed a mixed model using the R package 'lme4' (Bates 2010) where the response variable was a binomial measure of whether the applied fur clip was observed by the operator under field conditions. Age class of the badger (adult or cub) and sex were included as fixed effects as was the number of days since the mark was applied. Badger ID (as indicated by their tattoo) was included as a random effect to account for repeated observations of the same badger at different trapping events.

To test for variation in the size and depth of fur clips applied by operators to different age classes of badgers, we constructed a mixed model using the R package 'lme4' (Bates 2010) where the response variable was a binomial measure of whether the applied fur clip was 'small' (1), 'medium', or 'large' (0). Age class of the badger (adult or cub) was included as a fixed effect, and the operative who had applied the fur clip was included as a random effect to account for inter-individual variation in technique. A similar model was constructed to test whether fur clip depth varied between adults and cubs.

Detecting fur-clipped badgers using camera traps

In 2020, cameras (Bushnell Trophy Cam, Model 119,435) were deployed at ten badger setts in the study area where animals had previously been trapped and marked. Effort was concentrated at those setts with the most marked badgers and the motion-sensitive cameras were set to record 40 s long video clips once triggered, with a 20 s gap between recordings. Where available, two cameras were put out at a sett to account for technical failures.

Camera footage was reviewed to assess whether fur-clipped individuals could be clearly identified. We reviewed footage collected from approximately 1 h before sunset until approximately 1 h after sunrise. Following high levels of

camera failure in 2020, alternative models were trialled in 2021 (Browning Specs Ops Edge; Bushnell Core DS No-glow and Browning Recon Force Elite HP4). In 2021, cameras were deployed from 1st June (shortly after badgers were first marked on 26th May). During 2021, cameras were deployed at 11 setts where badgers had been fur clipped. Data from camera surveillance in both years were pooled for analyses. To assess the detectability of fur-clipped badgers, camera sessions (where a camera was deployed at a given sett for approximately 2 weeks) were restricted to those where any badgers (clipped or unclipped) were seen on screen. In order to assess the detectability of fur clips by remote surveillance, we constructed a mixed model in R (version 4.0.2) using the R package 'lme4' (Bates 2010). The response variable was a binomial measure of mark detectability (i.e. whether a camera deployed at a sett where badgers had been marked subsequently identified an animal with a clearly visible mark). The following fixed effects were assessed: camera effort (days a camera was operational in a given session × number of cameras deployed during that session), days since the mark was applied, and the number of marked individuals presumed to be present at a sett (estimated as the number that had been marked minus any known losses from the population). Interaction terms were not included because of the limited number of observations in the dataset ($n = 73$). Random effects were sett ID (to account for multiple camera sessions at the same sett) and the year of the study (to account for differences in camera model and performance between years). The significance of fixed effects was evaluated by stepwise model simplification using chi-squared test statistics and a threshold for p of 0.05. The over-dispersion function within the R package 'DHARMa' (Hartig 2020) was used to check the minimum adequate model, which indicated that it was not significantly overdispersed (dispersion test statistic = 1.0051, p-value > 0.05).

Results

Evaluation of livestock markers under field conditions

When tested on a clipped area of a badger pelt, all three products were readily visible, unfading, and durable in dry conditions. In 2020, a total of 57 individual badgers from 19 different setts in the study area were marked, 25 of which were marked under field conditions (i.e. the mark was applied to a conscious badger in a trap) and a further 32 during routine sampling under anaesthesia. We also trialled the use of battery-operated pet clippers as an alternative to scissors for applying a fur clip in the field, but this was quickly abandoned due to their poor performance on wet fur, the reaction of the badgers to the clipper noise, and difficulties in getting sufficiently close to the animal to use the clippers effectively and safely.

In terms of ease of application, both the marking paste and branding fluid products were straightforward to apply under field conditions. However, it was not possible to make a visible mark on the fur-clipped area using the livestock crayon under field conditions, so this option was quickly abandoned. The marking paste was used to mark 38 badgers: 14 under field conditions and 24 under anaesthesia with relatively balanced use across sexes (18 male, 20 female). Of the badgers marked using this method, eight were re-captured, and the average time interval between application and recapture was 65 days. The branding fluid was applied to a smaller number of badgers: eight in total (three male, five female) of which seven were marked in the field. Of the badgers marked using this method, three were re-captured, and the average time interval between application and recapture was 48 days. For both marking products, in all cases where marked badgers were recaptured and examined during sampling, the coloured marker had disappeared, although the fur clip remained visible, particularly on animals that were marked under anaesthesia rather than in the field.

Detection of fur clips by field operatives

During routine trapping operations in 2021, a total of 60 individual badgers were fur clipped under field conditions. The likelihood of a recaptured badger in a trap being correctly identified as having previously been marked was significantly negatively related to the number of days since the clip was applied ($\chi^2_1 = 13.43$, $P < 0.01$, see Fig. 1). No significant differences were noted in clip detectability between males and females ($\chi^2_1 = 0.59$, $P > 0.05$), but clips on cubs were significantly less likely to be detected at recapture events than those on adults ($\chi^2_1 = 5.38$, $P = 0.02$, see Fig. 1). The likelihood of having a small fur clip applied was significantly higher for cubs compared to adult badgers ($\chi^2_1 = 14.81$, $P = 0.0001$). On average, there was a 60% predicted probability of a cub being given a small fur clip under field conditions compared to 10% for adults. The likelihood of having a 'shallow' fur clip as opposed to a fur clip graded as 'medium' or 'deep' was significantly greater for cubs compared to adults ($\chi^2_1 = 4.45$, $P = 0.03$). On average, there was a 34% predicted probability of a cub being given a shallow fur clip under field conditions compared to 8% for adult badgers.

Detecting fur-clipped badgers using camera traps

The dataset for this analysis consisted of 73 surveillance sessions conducted in 2020 and 2021. The likelihood of a marked badger being captured by remote camera surveillance was significantly negatively related to the number of days since the mark was applied ($\chi^2_1 = 6.36$, $P < 0.05$,

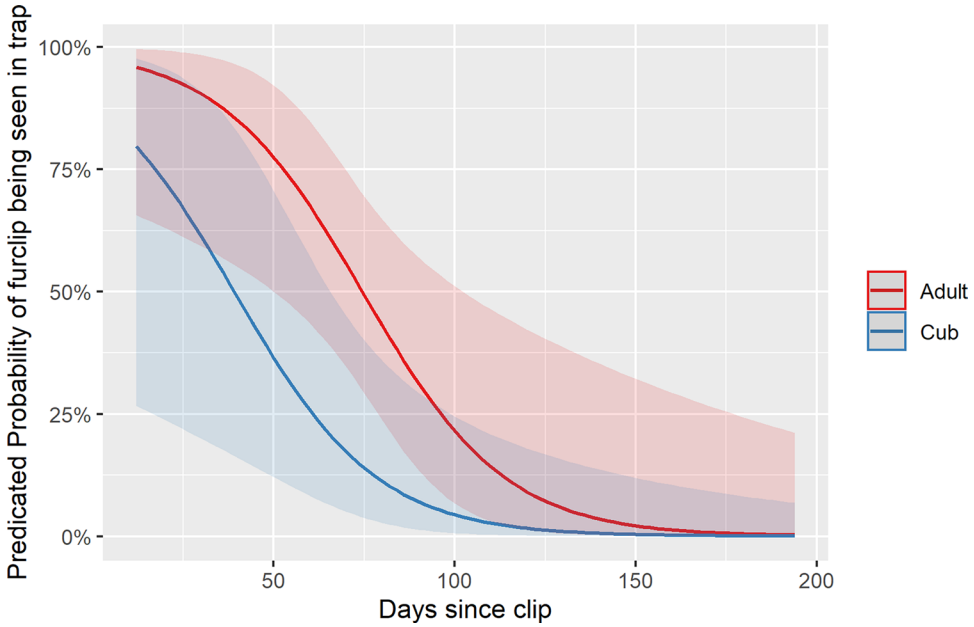


Fig. 1 Model-predicted decline in detectability of fur clips applied under field conditions over time for cubs and adult badgers. Note that the prediction lines commence from approximately 2 weeks after fur clip application as this is the earliest recapture interval represented in the dataset

see Fig. 2 below), consistent with marks becoming less detectable over time. The number of known marked individuals at a sett was significantly positively related to the probability that a marked badger would be detected by remote surveillance ($\chi^2_1 = 7.41$, $P < 0.01$, see Fig. 2). Camera surveillance effort was close to significantly associated with the likelihood of a marked badger being seen ($\chi^2_1 = 2.78$, $P = 0.09$). Examples of images of fur-clipped badgers collected by remote camera surveillance are shown in Box 2.

Discussion

In the present study, we evaluated the performance of methods of marking badgers that could be deployed in the field to identify animals that have been vaccinated. This would be particularly valuable in locations where badger culling was taking place in the proximity of vaccination operations. Under this scenario, whilst noting that the duration of the direct benefits of vaccination remains unknown, a vaccinated badger would ideally need to be readily identifiable for

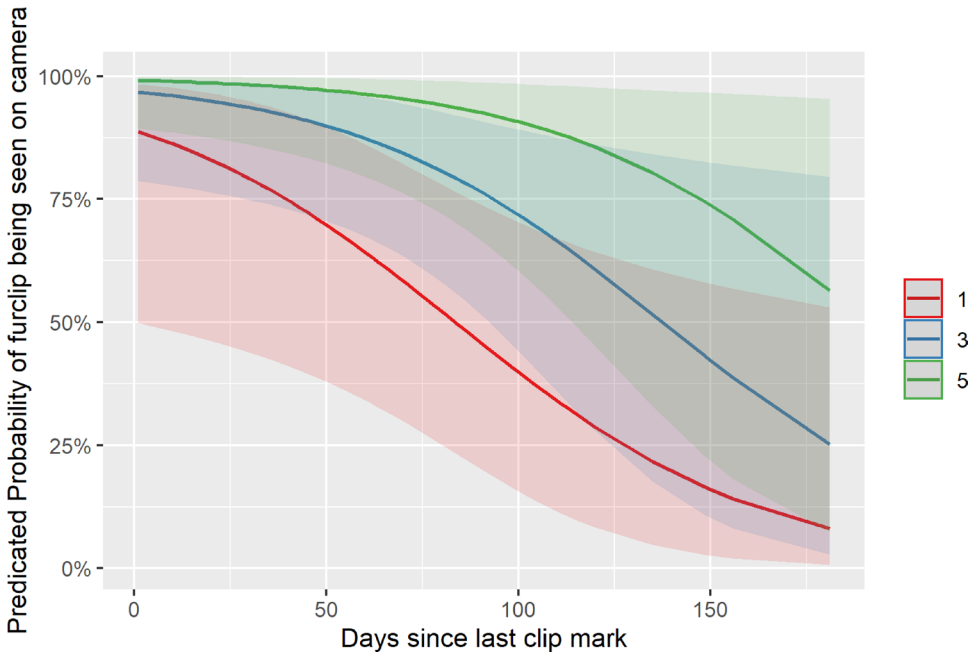


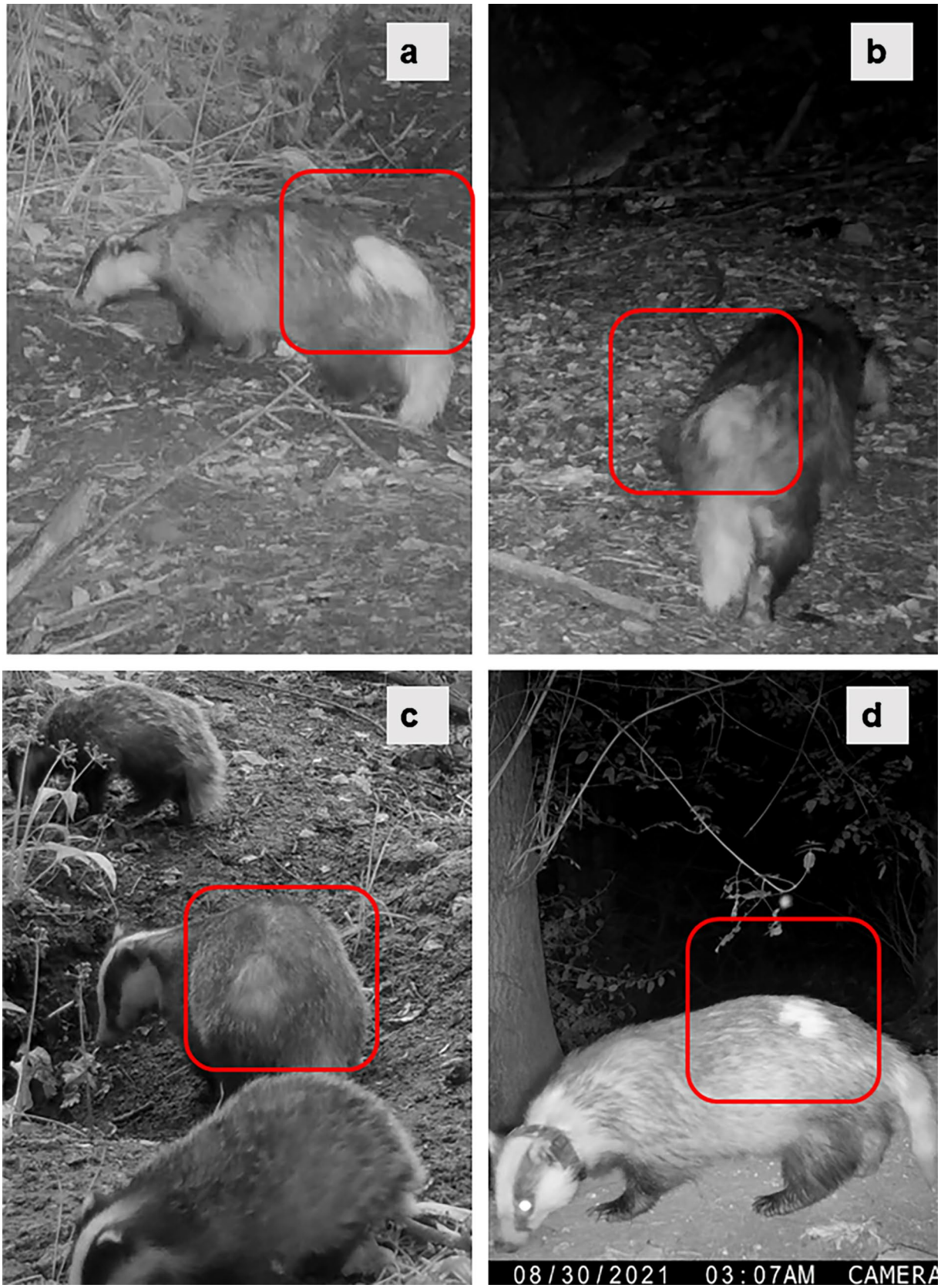
Fig. 2 Model-predicted decline in the likelihood of capturing images of fur-clipped badgers on remote cameras over time dependent on the number of fur-clipped individuals present at the sett (predictions shown for one (red), three (blue), and five (green) fur-clipped badgers). These predictions are based on an average camera surveillance period of approximately 2 weeks at any given sett. Note that the camera traps were activated shortly after badgers were marked at a given sett; hence, the prediction lines do not commence exactly at day zero

at least 12 months to reduce the risks of removing these benefits. The use of paints or marker products is preferable to more invasive marking methods due to their low cost, ease of application, and likely lower welfare impacts (Haines et al. 2018). However, finding suitably durable products for the intended purpose represents a practical challenge. This has been demonstrated in the present study where none of the products that were field-tested persisted well on badger fur. The products that were taken to field trial in this study were developed specifically for use on sheep, and hence physical differences between sheep wool and badger hair may at least in part account for their poor performance on badgers. It should however be noted that our search for a suitable marker was not exhaustive, and other suitable marker products that perform consistently (at least between annual moults) may be found or developed.

In contrast to a previous study where fur clips applied to anaesthetised badgers were detectable after 9 months on 80% of marked animals (Stewart and Macdonald 1997), our results suggest that 5 months after fur clipping, only about 5% of adult badgers and virtually no cubs would be correctly identified as having previously been fur clipped. The difference between these studies may relate to the method of clipping (anaesthesia permitting larger, clearer fur clips than is possible on conscious badgers), the size/shape of fur clip applied (marking under anaesthesia permitted the application of particular patterned marks on both sides of the badgers flank), and the conditions under which the marks were assessed for detectability (under anaesthesia in the 1997 study rather than under field conditions on conscious badgers in the present study). In the present study, we were not able to perform a direct comparison of the detectability of fur clips applied in the field as opposed to under anaesthesia as only a very small number of badgers were fur clipped under anaesthesia and subsequently re-caught. Also, importantly in the Stewart and Macdonald (1997) study, fur clips were applied in late August when adult badgers are more likely to have completed their moult, whereas they were applied in the largely pre-moult period of spring/early summer (Roper 2010) in the present study.

On the basis of the results of the present study, we would have very little confidence that fur-clipped badgers would be readily identifiable in cage traps for the entire trapping season (i.e. 1st May until 30th November in England (Natural England 2023)). Our model predicted that the detectability of fur clips on re-captured badgers under field conditions falls to 50% in adults by approximately 3 months post application. In badger cubs, the detectability of fur clips is far lower, falling to less than 50% within 6 weeks of the clip being applied. We suggest that there are two main drivers of the poorer performance of fur clips on badger cubs. Firstly, it has previously been documented that the period of rapid continuous fur growth in badger cubs means that fur clips quickly grow out and become undetectable (Stewart and Macdonald 1997). Secondly, we have demonstrated in the present study that badger cubs are significantly more likely to receive smaller, shallower fur clips. Anecdotal observations from experienced vaccinators suggest that (a) badger cubs tend to exhibit more agitated behaviour in the cage traps prior to vaccination and marking and (b) the smaller size of the cubs makes effective restraint (Natural England 2023) more challenging. This may be the reason why clips on cubs might be missed shortly after application as shallow and small marks might not be discernible from certain angles and/or once the coat hairs had resettled. In the context of identifying previously vaccinated badgers trapped during culling operations, the unreliable performance of fur clips on badger cubs is of concern as there would be a high risk of them not being identified and therefore being removed from the population. Removal of badgers (vaccinated or otherwise) from the population may result in perturbation effects (disruption of territories and enhanced movement of surviving animals; see Woodroffe et al. 2006) with the potential for counter-productive impacts on disease control. Also, the removal of vaccinated animals essentially cancels out the investment made towards herd immunity in badger populations as both the direct and indirect benefits of vaccination are lost. Annual vaccination of the new cohort of badger cubs recruited into the population is of key importance in terms of maximising disease control benefits as it is expected that cubs have lower likelihood of already being infected at the time of vaccination (the vaccine is not expected to have any therapeutic benefit in already infected animals) (Jenkins et al. 2008; Corner et al. 2012). However, expected TB prevalence differences between badger age classes are not always evident (Murphy et al. 2010; Sandoval Barron et al. 2018; Swift et al. 2021). The ambition to prioritise vaccination of badger cubs is balanced against their high mortality rate which has been estimated to be about 50% (Rogers et al. 1997). Hence, a substantial proportion of badger cubs vaccinated in the early part of the season (May/June) are unlikely to remain in the population in the longer term.

The results of the current study demonstrate the potential value of remote camera surveillance for determining whether recently vaccinated badgers are present in a given area, which may be useful when considering scenarios where culling and vaccination are taking place in close proximity. Although it is not practically realistic to apply unique fur clips (as used by Stewart and Macdonald (1997)) to conscious badgers through the bars of a cage trap, camera traps would have value in identifying whether vaccinated badgers were present at a given sett. As expected, the probability of a mark being detectable on surveillance footage declined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◂**Box 2** Examples of images of marked badgers captured by camera surveillance showing **a** an adult badger with a fur clip 1 month post application and **b** 2 months post fur clip being applied. **c** A detectable fur clip on a badger amongst unclipped individuals and **d** a marked badger also fitted with a radio collar as part of a separate research project.

over time, whilst the likelihood of a marked animal being detected increased with the number of individuals marked at any given sett. To provide some illustrative examples, if three badgers were trapped and vaccinated at a given sett in May, then our model predicts that a camera deployed there for 2 weeks in July would have an approximately 87% probability of detecting a marked badger. By August, this probability falls to about 75%, and from the end of October onwards, the probability of detection would be predicted to fall to around 25%. We therefore suggest that camera surveillance may provide a useful means of detecting the presence of previously vaccinated badgers at a given sett albeit only for a limited period and could not be relied upon to rule out the presence of vaccinated badgers over longer time intervals. Although data on where badgers have been vaccinated are already recorded (Defra 2022), this is only a single spatial point (i.e. where they were trapped), and badgers commonly move amongst setts. Vaccinated badgers may therefore turn up some distance from their original trapped location (Woodroffe et al. 2017); hence, camera traps may be a useful means of detecting their presence in areas spatially proximate to where vaccination has taken place. Badger vaccination is increasingly being deployed in England in populations which have recently been culled (Benton et al. 2023), in line with the stated government policy direction (Defra 2020b). Culling has been demonstrated to impact on the behaviour of surviving badgers, characterised by larger home range sizes (Woodroffe et al. 2006; Pope et al. 2007; Ham et al. 2019). Where badger vaccination is taking place in this context (i.e. post-cull) and culling may be continuing in adjacent areas, the use of remote cameras may provide valuable information on the likely risks of vaccinated badgers being removed as they move between land parcels under different management interventions.

Results of the present study suggest it is highly likely that, if badger vaccination and badger culling were carried out in adjacent areas within the same trapping season (i.e. between May and November of a given year) and fur clipping was the primary means of marking, then a proportion of vaccinated badgers would likely be removed, in particular badger cubs. In terms of mitigating this risk, buffer zones have been proposed previously, consisting of voluntary 'no-cull' zones surrounding badger vaccination sites. However, it has been suggested that precluding the surrounding badgers from either vaccination or culling may adversely impact disease control efforts (Defra 2020a). An alternative solution may be to optimise the timing of vaccination and culling operations to minimise potential losses of vaccinated badgers. This could include restricting culling to the use of cage trapping rather than shooting within a given distance of a vaccination site (as proposed previously (Defra 2020a, b)) which would provide the best opportunity for spotting any marked animals. Another measure would be to ensure that culling took place within a short time period (for example 1 month) of vaccination which would be within the likely window of duration of fur clips. Alternatively, management approaches could be temporally distanced, for example in a scenario where a central cull area was surrounded by ring vaccination, only carrying out culling in the central area in the year following vaccination may potentially reduce the risks of removing recently vaccinated badgers. However, as the duration of both the direct and indirect benefits of vaccination in badger populations remains unknown, the implications of such a strategy on disease transmission within badger populations and, crucially, between badgers and adjacent livestock are unclear. We recommend further simulation studies (Smith and Budgey 2021) to model the impacts of such combined management interventions on disease transmission within badger populations and onward risks of transmission to cattle. This would inform how management approaches might be optimised such that disease control benefits are maximised whilst balancing resource-related trade-offs including how frequently an approach is deployed and the spatial scale at which an intervention is feasible.

In England, it has been acknowledged that 'the co-existence of vaccination and culling needs to be carefully managed to facilitate deployment of both control methods in a complementary manner' (Defra 2020a). The findings of this study suggest that this presents a particular practical challenge where the intention is to use vaccination in close proximity to culling and that fur clipping is the only currently available option for marking vaccinated badgers. Permanent identification methods which require anaesthesia, such as microchipping, have challenging resource implications which may impede the process of vaccination deployment in the field resulting in poor vaccine coverage. In addition, where the duration of immunity is unknown (as in the case of badgers and the injectable BadgerBCG vaccine), permanent identification of vaccinated individuals may serve limited purpose as there is no expectation that a single dose invokes lifelong immunity, and therefore knowing that a badger had already been vaccinated in the previous year would not affect the decision to re-vaccinate. There are examples of research applications where badgers have been anaesthetised and microchipped within the context of a vaccination research project, leading to valuable insights on the impacts of a single vaccine dose in badgers (Aznar et al. 2018) and individual-level behavioural responses to

vaccination (Woodroffe et al. 2017). However, in the context of operational deployment of badger vaccination aiming to cover as large an area as efficiently as possible, the additional time (and specialist training) required to anaesthetise and microchip every trapped badger would undoubtably limit spatial coverage substantially. Furthermore, field anaesthesia is not without its risks for the subject animal, with the potential for post-release impacts being generally poorly understood (Colloff et al. 2024). Other options for individual identification, such as collecting and genotyping hair samples from trapped badgers without anaesthesia (Smith et al. 2020), may lend themselves better to an operational context as they are not so time-consuming. Whilst this would not permit trap-side identification owing to the subsequent time required for genotyping, it would allow quantification of how many doses a badger receives during a multi-year vaccination campaign, more accurate estimation of the proportion of the population vaccinated (Benton et al. 2020), and assessment of genetic population structure and health (for example levels of inbreeding) in recovering badger populations.

Outside the UK, whilst the majority of research effort continues to be directed towards the development of an oral vaccine for wildlife (Buddle et al. 2018), the use of an injectable BCG vaccine is an area of international research interest in cervids, wild boar, and brushtail possums (see review in (Balseiro et al. 2020). Using practical lessons learnt during the ongoing deployment of a TB vaccine, our study highlights the need to explore combined approaches to disease control in wildlife at different spatial scales and hence to develop the necessary practical tools to optimise effectiveness.

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Author contributions C.H.B and R.J.D wrote the manuscript. A.G project managed data collection for all field data and catalogued and collated all data for onward analyses. C.H.B carried out all statistical analyses. All authors reviewed the manuscript.

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Availability of data and materials We intend to archive the dataset used for the analyses on the Data Dryad Repository should this paper be accepted for publication. We also intend to archive relevant camera images on the MammalWeb repository (Home (mammalweb.org)) following publication.

Declarations

Ethical approval Badgers were trapped under a Natural England Science and Conservation licence (licence reference covering the period of this study: 2020-47090-SCI-SCI-1). Cage traps used for capture were of an approved design under the AIHTS (Agreement on International Humane Trapping Standards) regulations (EU 2019). Consultation on the use of the livestock markers in field trials took place with the APHA Animal Welfare and Ethical Review Board (AWERB) and the UK regulatory authority (Home Office), and relevant approvals were obtained prior to the commencement of field trials.

Competing interests The authors declare no competing interests.

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