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Evaluating seasonal bait delivery to badgers using rhodamine B

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Abstract In the UK and Ireland, research on the control of bovine tuberculosis in badgers includes the development of a palatable bait for oral delivery of a vaccine and a means of its deployment in the field. In the present study, we carried out field deployment of bait according to the established method of bait marking in early spring and early summer to compare the effects of seasonality on bait uptake rates. All baits contained rhodamine B (RhB) which was subsequently detected in the hair and whiskers of captured badgers. During the 8 days of bait feeding at 14 badger setts, 99% of baits deployed in spring, and 100% of those deployed in summer were removed. The presence of RhB in captured badgers indicated high rates of uptake amongst adult badgers in spring (93%) and summer (98%). Only cubs captured in summer showed evidence of having taken bait (91%). Between 67% and 100% of each social group was estimated to have taken bait. The detection of RhB in 96% of badgers captured at outlier setts, where bait was not fed, suggested that deployment at main setts alone may be sufficient to target a relatively high proportion of the badger population. The number of baits deployed per marked badger suggested that a similar level of uptake might be achievable using fewer baits. The results clearly

demonstrate the potential value of the bait-marking methodology for delivering vaccine baits to badgers during spring and summer, but that deployment in early summer is necessary to target cubs.

Keywords Vaccine bait · Deployment · Biomarker · Season · Wildlife vaccination · Bovine tuberculosis

Introduction

In the UK and Ireland, the Eurasian badger (*Meles meles*) is a source of *Mycobacterium bovis* (the causative agent of bovine tuberculosis (bTB)) infection in cattle (Krebs et al. 1997). Previous attempts at managing transmission to cattle by culling badgers have met with mixed success (Griffin et al. 2005; Donnelly et al. 2006), and recent research efforts have focussed on the vaccination of badgers as a long-term solution. The deployment of baits containing vaccine has been the principal method for the successful management of rabies in foxes throughout much of Western and Central Europe (Flamand et al. 1992; Stöhr and Meslin 1996; Cliquet and Aubert 2004). More recently, oral vaccination has been employed in field studies of the management of bTB in brushtail possums (*Trichosurus vulpecula*) (Tompkins et al. 2009). In the UK and Ireland, much current research effort has been focused on the development of a palatable bait for the delivery of live BCG (Bacille Calmette Guerin) to badgers and a means of deploying it to a high (but as yet undefined) proportion of the badger population.

The effectiveness of a field vaccination programme is dependent on the proportion of susceptible individuals that receive the vaccine and the proportion that become immunised (Delahay et al. 2003). In the case of vaccine

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delivery in oral baits, the proportion of the target population that will consume deployed bait will be critical in determining success. Badgers may be a promising candidate for delivery of vaccines in oral bait as they live in groups in conspicuous underground dens (setts) which can be targeted for bait delivery. Furthermore, badgers have a varied diet (see Delahay et al. 2008) which provides opportunities to investigate a range of potentially palatable types of bait. Several studies have investigated bait consumption by badgers, with reported uptake rates in subsequently trapped animals of 22% for meat, 31% for fruit- and 35% for cereal-based baits (Cagnacci et al. 2007), 81.5% and 69% for commercial fish-based baits (Southey et al. 2001, 2002, respectively) and 96% for peanuts and syrup (de Leeuw et al. 2006).

The timing of bait deployment will also affect the success of a vaccination programme as optimum uptake is likely to be dependent on the availability of naturally occurring food, which in turn may be influenced by season and weather conditions (e.g. Kruuk and Parish 1985). Furthermore, the timing of bait deployment will determine whether vaccine is delivered to susceptible juveniles. In the UK, most badger cubs are born in February and emerge above ground after approximately 8 weeks (Delahay et al. 2008), indicating that early spring may be the optimum season in which to deliver vaccine baits. However, a number of constraints may affect the availability of baits to cubs at this time of year. First, cubs are relatively small upon emergence and are likely to be prevented from accessing food resources by older and more dominant animals. Second, in order to deter non-target species, baits deployed at badger setts are usually placed in shallow depressions under large stones (see Delahay et al. 2000), which may also make them less accessible to small cubs. Placing baits down badger sett entrance holes would, however, not disperse them sufficiently to reduce the chances of monopolisation by a relatively small number of adult animals. Therefore, in order to maximise bait uptake by cubs, it may be advantageous to deploy oral vaccine baits above ground in late spring or early summer when cubs are larger and able to forage independently.

A number of studies have used chemical biomarkers to monitor bait uptake for orally delivered vaccines and poisons (Morgan 1982; Trehwella et al. 1991). When incorporated into bait, such markers may allow the identification of individuals that have consumed bait through examination of either body fluids or tissues. In previous studies, iophenoxic acid (IPA, e.g. Southey et al. 2001; Cagnacci et al. 2006; de Leeuw et al. 2006; Cagnacci et al. 2007) and rhodamine B (RhB, e.g. Cagnacci et al. 2006, 2007) have been successfully employed to measure bait uptake in badgers. IPA is relatively expensive to purchase and process and involves obtaining a blood

sample and detection using high-performance liquid chromatography (see Jones, 1994). In contrast, RhB is relatively cheap and can be detected by simply looking for stained sections of hair or whiskers using fluorescence microscopy. The persistence of IPA (a serum marker) and RhB (a marker of keratinous tissue) in badgers appears similar, having been detected at up to 20 and 24 weeks post consumption, respectively (Cagnacci et al. 2006). However, a distinct advantage of using IPA as a biomarker is that unlike RhB, some of its analogues can provide quantitative information on the amount of bait consumed. Nevertheless, low relative costs and simplicity of application make RhB an attractive option for the investigation of certain aspects of bait uptake in badgers.

The aims of the current study were to provide information to help support the development of protocols for the deployment of TB vaccine to badgers in the form of an oral bait. First, we sought to assess the value of using the systemic biomarker RhB in future investigations of bait uptake by badgers. Previous work (de Leeuw et al. 2006) has shown that deploying a highly palatable bait in the field according to the established method of bait marking (see Delahay et al. 2000) is an effective means of delivering it to a high proportion of the population. This deployment protocol is used for research purposes to delineate the territorial configuration of badger social groups and may serve as a useful model on which to base a strategy for the delivery of a bait containing vaccine. In the present study, we fed bait in early spring (as per de Leeuw et al. 2006), when territorial activity is considered to be at its peak (Kruuk 1978; Roper et al. 1993) and when bait marking is usually employed and repeated the procedure in early summer. This allowed us to compare the effects of timing of bait deployment on bait uptake rates in badgers, and in particular, to assess the effectiveness of summer deployment for targeting cubs. Finally, this investigation also provided data to allow initial estimations of the effort (and therefore costs) likely to be involved in delivering vaccine in an oral bait to a given proportion of a badger population. Such information is one crucial component in assessing the economic viability and cost-effectiveness of this strategy for disease control.

Methods

Study area and badger population

The studies were carried out in Woodchester Park, Gloucestershire, southwest England (51°43'N, 2°16'E) in spring (February) and summer (June) 2008. The study area is approximately 11 km² and consists predominantly of a central steep-sided wooded valley surrounded by farmland

(see Delahay et al. 2006). A regular capture-mark-recapture programme has been carried out at the site since 1981 for the purposes of investigating badger ecology and the epidemiology of *M. bovis* infection. Each badger social group was routinely trapped four times a year, and the spatial configuration of group territories was determined annually in spring by bait marking (Delahay et al. 2000). The latter involved feeding bait laced with indigestible plastic markers at every potential main sett in the study area and then surveying the area to locate latrines and identify the plastic markers.

As each badger was given a unique tattoo when first trapped (Cheeseman and Harris 1982), they could be identified during subsequent recaptures. This allowed us to use observations of badgers captured in our target social groups in 2007 and 2009 to calculate Minimum Number Alive (MNA) estimates of group sizes in 2008. This is important because in the present study, we were unlikely to capture all the residents of a given social group during the one trapping session at which we tested for the presence of biomarkers.

Bait preparation and deployment

The bait routinely used for the annual spring bait-marking exercises consisted of peanuts, golden syrup and coloured plastic beads. Bait was usually prepared in small batches in the morning of the day of intended use. Each portion of bait consisted of approximately 100 ml of the mixture (Delahay et al. 2000). Hence, 2 kg of peanuts, 250 g of plastic beads and 250 ml golden syrup mixed in a 10-l bucket provided approximately 20 portions of bait. For the purposes of the present study, 14 setts were selected from the total of 26 putative main setts in the study area. Bait fed at the selected setts also contained RhB (Sigma-Aldrich, Dorset, UK). The recommended RhB dose per bait was 100 mg (Cagnacci et al. 2007) and so 2 g (i.e. 20×100 mg) of the marker was added to each bucket of 20 baits. The appropriate dose of RhB was weighed and admixed with warmed golden syrup before being poured over the peanut mixture. Because RhB turns pink upon contact with moisture, staff could see when the dye was evenly mixed. During deployment, staff ensured that approximately 20 baits were taken from each prepared bucket. Using the same approach (although without the addition of coloured plastic beads) the same 14 setts were once again fed with bait containing RhB during early summer.

Bait containing RhB was deployed at each of the 14 selected setts for eight consecutive days, starting on the 15th of February during the spring deployment session and on the 15th of June in the summer. In each case, this was preceded by a short period of 'pre-baiting' during which time the same bait, but without RhB, was placed directly

down sett entrance holes for 3 days in spring and 2 days in summer. This pre-baiting period is standard practice during bait-marking as it allows the resident animals to become familiarised with the availability of a new food source (Delahay et al. 2000). During this period, rates of bait removal could not be quantified. Following pre-baiting, baits were placed above ground daily for 8 days, each in a shallow depression covered with a heavy stone to discourage non-target species. The broad aim of bait deployment was to ensure that every distinct badger path leading away from the sett entrance holes had at least one bait point on it. Hence, the number of baits deployed per sett each day varied from 8 to 35 (mean 19.7 ± 0.4) depending on the size of the sett and the number of paths. Bait points were moved every few days in an attempt to reduce monopolisation, which might be more likely if certain individuals became familiar with their location. The number of baits that were deployed and removed was recorded on a daily basis.

The total amount of RhB deployed over the 8-day period was approximately 6.4–28 g per sett. The average weight of badgers trapped at the fed setts throughout the duration of the study was 8.3 kg (spring=8.35 kg, summer=8.29 kg). Therefore, if a single animal consumed all the bait available at a single sett (a highly unlikely scenario), the maximum daily dose of RhB received would range from 96.4 to 421.7 mg/kg. This is substantially less than the oral LD50 for mice (887 mg/kg; Sigma-Aldrich 2006). In addition, as RhB is rapidly metabolised, repeated exposure during the 8 days of feeding would be highly unlikely to result in significant accumulation (Fisher 1999). However, RhB has been observed to exhibit a high degree of persistence in keratinous tissue (Fisher 1999) and is detectable (at the same dose of 100 mg per bait) in badger whiskers up to 24 weeks (168 days) after ingestion (Cagnacci et al. 2006).

Capture and examination of badgers

For practical reasons, the Woodchester Park study area is split into three zones for the purposes of the routine-trapping programme. The badger setts selected for the present study were located in zones A ($n=8$) and B ($n=6$). In spring, badgers in zone A were trapped on 6th and 7th May (74 days after bait feeding), whilst those in zone B were trapped on 20th and 21st May 2008 (88 days after bait feeding). In summer, badgers in zone A were trapped on 22nd and 23rd July (30 days after bait feeding), whilst those in zone B were trapped on 5th and 6th August 2008 (44 days after bait feeding). No badger trapping took place from February to May inclusive (the 'close season') as this is when most cubs are born and females are suckling-dependent young (see Woodroffe et al. 2005). Although the interval between bait deployment and trapping differed between spring and summer, this should not have influ-

enced the results, as in both cases, animals were captured and sampled well within the 168-day period during which RhB is known to be detectable in badger whiskers (Cagnacci et al. 2006). The shortest interval between bait deployment and trapping was 30 days, which was enough time to allow whiskers to grow sufficiently to be able to detect RhB staining in the shaft (Spurr 2002).

As part of the routine-trapping programme, badgers were captured at the setts where marked bait was fed and also at other (outlier) setts within the associated social group territories. Steel mesh box traps were located on or near to badger ‘runs’, baited with peanuts and set after 4–8 days of pre-baiting (Cheeseman and Mallinson 1979). Captured badgers were transferred to holding cages and transported back to a central location for anaesthesia (de Leeuw et al. 2004) and examination.

The longest whisker from each side of the snout (i.e. two in total) was removed from each captured badger, cutting it as close as possible to the skin. In addition, a small number of hairs (approximately 10) were plucked from the nape, flank and rump of each animal and stored separately in airtight bags. No whiskers were taken from cubs in the spring for welfare reasons and because they would not be expected to have had access to baits deployed in February as they would still be confined underground at that time (Delahay et al. 2008). In addition, although whiskers are considered more reliable than pelage hair for detecting RhB owing to their near-continuous growth phases (Fisher 1999), staining has been detected previously in the guard hairs of badgers (Southey et al. 2002). Body hairs were only examined in the event that the whiskers were negative for RhB staining. All whisker and hair samples were stored in a dark airtight container to prevent deterioration of any RhB staining by UV light prior to examination. Whiskers and hairs were observed at $\times 10$ magnification using a compound microscope fitted with filters compatible for use with UV light and rhodamine probes (Leica DMLB, Wetzlar, Germany). The presence of RhB was indicated by a bright fluorescent band at some point along the shaft of the whisker (Fisher 1998). The spring and summer bait-feeding exercises would be expected to result in separate bands in the whiskers (see Purdey et al. 2003) given their rate of growth and the time interval between bait deployment sessions (114 days from the last day of bait feeding in spring to the start of bait deployment in the summer).

Statistical analysis

Descriptive statistics were used to summarise bait removal rates and the presence of RhB in trapped badgers. Logistic regression (see Collett 1999) was used to investigate variations in bait removal rates and the presence of RhB (dependent variables). Variations in bait removal rates were

related to season and sett. Variations in the presence of RhB in badgers were related to season, sex, age and social group.

We assumed that the sample of badgers that we trapped for the purposes of this study was representative of the wider population on the study site and hence that we could extrapolate the proportion in which biomarker was detected to the entire social group. The total number of badgers that we assumed had taken bait (i.e. including animals that were not subsequently trapped) was therefore approximated by multiplying the MNA estimate of social group size by the proportion of trapped animals in which biomarker was detected. We then divided the number of baits that had been deployed by the estimated total number of badgers that had taken bait. This provided an indication of the average number of baits that needed to be deployed per badger to achieve the observed level of uptake under the prevailing circumstances.

Results

General

The bait-marking exercise in spring 2008 involved feeding marked bait at 26 putative main setts, 14 of which were selected for inclusion in the present study. Subsequent examination of latrine survey data (see Delahay et al. 2000) indicated that these 14 setts corresponded to 11 different social groups (i.e. three social groups contained two putative main setts).

Bait removal rates

During the 8 days of bait feeding, 2,155 (98.8%) of the 2,181 baits deployed in spring (February) were removed, and in summer (June), all 2,228 baits were removed. Hence, in total, only 26 of 4,409 (0.6%) deployed baits remained in situ when feeding ended, although these were eaten during subsequent days. The proportion of baits that were removed daily throughout the course of the 8-day feeding period varied significantly between seasons ($F=45.3$, d.f.=1, $p<0.001$), with higher removal rates in summer than in spring. There was also a significant difference in the daily proportion of removed baits between setts in spring (from 96.5% to 100%; $F=40.6$, d.f.=13, $p<0.001$), whereas in summer, daily bait removal was consistently high (always $p>0.05$).

Bait biomarker uptake

A total of 54 adults and nine cubs were caught in May 2008, following bait deployment in spring (Fig. 1). All

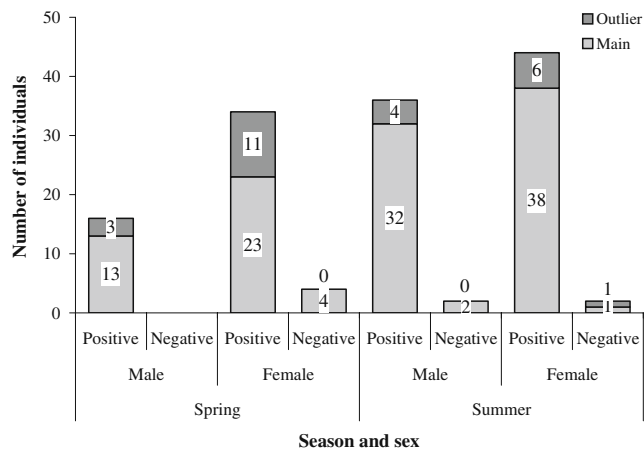


Fig. 1 The total number of badgers captured in spring and summer 2008 within the territories of social groups where bait was fed. The number of animals that took bait (positive) was indicated by the presence of rhodamine B staining in whiskers and/or hair. Note that the nine cubs captured in spring have been omitted as they were yet to be weaned and emerge above ground at the time when bait was fed

body hairs taken from cubs in May were negative for the presence of RhB. Fifty (93%) of the 54 adult badgers captured in the territories of those social groups where marked bait was fed had RhB staining either in whiskers (49) or hair (1 additional animal). Fourteen (26%) of the 54 adults were caught at outlier setts and all were RhB positive. Thirty-four (90%) of the 38 females and all of the 16 males trapped were RhB positive, with no significant difference between the sexes ($F=2.9$, d.f.=1, 49, $p=0.1$). The four females that tested negative for RhB were all trapped at main setts where bait was fed, where they had previously been trapped several times prior to the current study.

A total of 49 adults and 35 cubs were caught in July and August following bait deployment in June (Fig. 1). Overall, 80 (95%) of the 84 trapped badgers had RhB staining in either the whiskers (78) or hair (two additional animals). This equated to 98% of the adults and 91% of the cubs that were trapped. The single adult with no RhB staining was a female, which was captured at one of the main setts where bait had been fed and had been previously trapped there several times prior to the current study. Sixteen (94%) of the 17 female cubs and 16 (89%) of the 18 male cubs trapped were RhB positive. There was no significant difference in the presence of biomarker between the sexes (approximately $F=0$, d.f.=1, 83, $p=0.96$) or age categories ($F=2.0$, d.f.=1, 83, $p=0.2$). Eleven (13%) of the 84 badgers caught in July and August were captured at outlier setts where bait was not fed, 10 (91%) of which tested positive for RhB staining. Three of the four RhB negative badgers were trapped at setts that were fed bait. All three badgers were classed as resident, having been trapped either several times before (adults) or several times after (cubs) the current study in their respective social groups.

The presence of RhB in trapped badgers did not vary significantly between seasons ($F=1.7$, d.f.=1, 134, $p=0.2$), and there was only a slightly higher percentage of individuals testing positive for the marker in summer. Thirty-two badgers (24 adults and eight cubs, 28% of the total 115 individuals caught) were caught in both the spring and summer. No cubs were RhB positive in the spring (see above) but seven of eight that were caught in both seasons were RhB positive in summer. All of the adult badgers caught in both seasons were positive for RhB on both occasions, except two, one of which was positive in spring only, and one which was positive in summer only. Bands of rhodamine staining in the whiskers of badgers that took bait in the spring had usually grown out by the time they were recaptured after consuming marked bait in the summer. Consequently, only two of the 24 adult badgers known to have consumed bait in both seasons had two distinct bands in their whiskers at 22 weeks after bait was fed in the spring.

MNA estimates of social group size (mean=11.3, range=5–17) did not differ between seasons as they were derived from the same data. The proportion of trapped badgers at each social group (and the assumed proportion of the entire social group) in which RhB was detected varied between 67% and 100% in both seasons (Table 1). The number of deployed baits per RhB marked badger was similar in both seasons, although the range of values per social group was wider in spring (Table 1).

Discussion

The results from the current study show that the bait-marking methodology used for territory mapping (Delahay et al. 2000) may constitute a useful approach for delivering vaccine baits to a high proportion of a badger population during either spring or summer. However, bait deployment in February would not be appropriate in order to target cubs. These results also illustrate the current and likely future value of RhB as a biomarker for investigating bait uptake in support of studies to develop effective TB vaccine deployment strategies.

The attractiveness and palatability of bait are major factors affecting rates of uptake by badgers (see Southey et al. 2001; Cagnacci et al. 2007). Practical experience during the long-term studies at Woodchester Park has established that the peanut and syrup mixture routinely used during bait-marking exercises is highly attractive to badgers. Results from the present study showed that 93% of captured adult badgers in spring and 95% of those captured in summer had consumed RhB-laced bait. This is comparable with previous work (de Leeuw et al. 2006) which demonstrated uptake rates of 96% during spring using the

Table 1 The number of baits deployed within social groups, numbers of badgers trapped and those that were positive for rhodamine B, a Minimum Number Alive (MNA) estimate of the total social group size and the mean number of baits per biomarker positive badger in (a) spring and (b) summer

Social group	Number of baits deployed	Number trapped	Number (%) positive	MNA estimate of social group size	Estimated mean number of baits per badger
(a) Spring					
West	196	4	4 (100)	8	24.5
Larch*	287	5	4 (80)	11	32.6
Hedge	212	5	5 (100)	17	12.5
Junction*	278	5	5 (100)	10	27.8
Jacks	144	6	6 (100)	12	12.0
Arthurs	100	3	3 (100)	12	8.3
Beech	192	5	5 (100)	16	12.0
Septic tank	115	3	3 (100)	8	14.4
Yew*	333	9	6 (67)	14	35.7
Wych Elm	175	5	5 (100)	11	15.9
Top	142	4	4 (100)	5	28.4
Total	2,181	54	50 (mean=95%)	124	Mean=20.4
(b) Summer					
West	221	3	2 (67)	8	41.4
Larch*	270	9	9 (100)	11	24.5
Hedge	153	11	10 (91)	17	9.9
Junction*	236	10	10 (100)	10	23.6
Jacks	132	8	8 (100)	12	11.0
Arthurs	112	9	8 (89)	12	9.3
Beech	215	12	12 (100)	16	13.4
Septic tank	155	3	3 (100)	8	19.4
Yew*	361	6	6 (100)	14	25.8
Wych Elm	241	8	7 (88)	11	25.0
Top	132	5	5 (100)	5	26.4
Total	2,228	84	80 (mean=94%)	124	Mean=20.9

The mean number of baits per badger was calculated from the total number of baits deployed (see text). Note that the nine cubs captured in spring have been omitted as they were yet to be weaned and emerge above ground at the time when bait was fed. Asterisks identify social groups where more than one putative main sett was fed

same bait (but with an IPA biomarker) deployed according to the bait-marking methodology. However, the composition of the bait used in the present study is likely to differ from that of the final choice for the delivery of an oral vaccine as the latter will also need to satisfy several other criteria (e.g. compatibility with the vaccine and environmental persistence).

The success of a badger vaccination strategy will be dependent on the number of badgers taking bait and the efficacy of the oral vaccine once consumed. There are several reasons why estimates of bait uptake from the present study are likely to represent ‘best-case’ scenarios. Firstly, badgers in the Woodchester Park study area are frequently exposed to peanuts (during trapping) and the peanut and syrup bait (during bait marking) so they are likely to exhibit lower levels of neophobic behaviour than

bait-naive populations. Consequently, the present study provides estimates of bait uptake that can be achieved under ideal conditions. In addition, we cannot assume that all the resident members of each badger social group will have been captured during our study. We tried to minimise this effect by using an MNA estimate of social group size for some calculations, which took into account trapping events before and after this study took place. Nevertheless, some badgers may consistently avoid capture, and these animals may also have been less likely to take baits deployed in the present study. Consequently, our approximations of the proportion of badgers in each social group which took bait may be over-estimated. However, this effect is likely to be relatively small as previous analyses of data from this population indicate that a high proportion of the resident badgers are trapped and recapture rates are high

(Rogers et al. 1997; R. Delahay, unpublished data). This suggests that we can have some confidence in the relatively high levels of bait uptake reported in this study and in the conclusion that this is likely to be a useful approach for the delivery of vaccine baits to badgers.

Previous trapping data indicated that a high proportion (88%) of the small number ($n=8$) of badgers that tested negative for RhB in the present study were likely to have been in residence in the targeted social groups at the time of bait deployment. Given the high levels of bait provision at main setts, it is not clear why these animals failed to consume any, although it is possible that they were more neophobic or less competitive than other badgers, or they may have been occupying an outlier sett throughout the feeding period. Alternatively, the two whiskers selected for sampling from these individuals may not have been marked with RhB whereas other whiskers were, although the very high rates of staining detected in trapped badgers suggests that such an effect was likely to have been minimal. Nevertheless, if animals that had consumed bait had remained undetected as a result of this approach, then the true rate of bait uptake in captured animals may have been even higher.

The timing of deployment of vaccine baits may be crucial in determining the success of the vaccination campaign, as bait uptake may vary with respect to the availability of natural foods. In the current study, early spring and summer were selected because bait uptake was expected to be highest during these two seasons in comparison to autumn and winter. In winter, badger activity levels are significantly reduced in response to a variety of social and environmental factors, and in autumn, the availability of natural foods is high (Neal and Cheeseman 1996), so in both instances, bait uptake would be expected to be relatively poor. In contrast, the likelihood that bait will be taken may be enhanced as levels of activity increase in late winter/early spring as young cubs are reared, females enter post-partum oestrus and there is an increase in territorial activity (Kruuk 1978; Roper et al. 1993). In summer, warmer and drier weather conditions reduce the availability of preferred food items such as earthworms (Kruuk and Parish 1987), which is also likely to be conducive to higher levels of bait uptake. The results from the current study showed similarly high levels of bait removal and biomarker presence in trapped badgers in both spring and summer, although bait removal rates were significantly higher in summer. However, despite the method of bait marking being designed to reduce opportunities for non-targets taking bait (by deploying it under stones), all bait removal cannot unequivocally be ascribed to badgers. In our study area, video surveillance from other studies (K Palphramand, unpublished data) and anecdotal reports of field signs suggest that most non-target uptake is

probably related to small mammals, which are unlikely to remove an entire bait portion on a given night. Consequently, the impact of non-target bait uptake on the present study was likely to have been minimal. Also, daily removal rates provide no information on the number of badgers that may have taken baits. As there was no significant seasonal difference in the proportion of trapped badgers that tested positive for RhB (indicative of having consumed at least one bait), this suggests that any seasonal differences in bait removal rates do not correspond to seasonal differences in the proportion of badgers that took bait. Significant variations in daily removal rates amongst setts in spring could potentially be related to spatial variations in the activity of non-target species or the feeding behaviour of the resident badgers at this time of year.

Bait uptake rates have been shown to be high in spring using the bait-marking approach (de Leeuw et al. 2006), but the additional deployment of bait in summer in the present study provided the opportunity to assess the potential to deliver vaccine to susceptible cubs. The capacity to target and immunise susceptible individuals, such as cubs, as early as possible during their life could potentially have a significant impact on the likelihood of success of a vaccination campaign (Delahay et al. 2003). For example, the results of field investigations of classical swine fever in wild boar populations suggest that piglets play an important role in the persistence of infection, particularly in vaccinated populations, as they do not readily take vaccine baits (S. Rossi, personal communication). In the present study, badger cubs were highly unlikely to have had access to baits deployed in mid to late February as at this time of year, the overwhelming majority will have been neonates yet to emerge above ground or begin weaning (Delahay et al. 2008). Transfer of RhB from lactating females to their cubs has not been demonstrated but is theoretically possible as this compound is highly water soluble (Fisher 1999), although it is not known whether this would have resulted in detectable staining in badger hair in the present study. We did not detect biomarker in any hair samples collected from cubs trapped following the February deployment of bait in this study. However, bait deployment in early summer yielded evidence of uptake in 91% (32 of 35) of cubs that were subsequently captured. Although adults would have a competitive advantage in terms of taking bait at this time of year, there was no significant difference in the presence of RhB between age categories. This is likely to be attributable to the method of deployment employed during bait marking, which ensures that baits are sufficiently widely broadcast to reduce opportunities for bait monopolisation.

The bait-marking method uses a saturation approach to bait deployment to ensure that sufficient quantities of plastic markers are found in faeces during the subsequent

latrine survey (Delahay et al. 2000). Hence, more bait is generally deployed at a given sett than the number of badgers anticipated to be present, although this is a subjective judgement. Bait uptake is further maximised by feeding for eight consecutive days. Data from the present study suggest that on average, this level of bait provision resulted in one marked badger per approximately 20 baits deployed. Clearly, there is some scope to optimise the approach such that fewer baits are deployed to achieve similarly high levels of uptake. This will be an important consideration for a vaccination strategy where the cost of individual bait portions may impose limitations on what is cost-effective. Future work is planned to optimise bait deployment by investigating how the proportion of the population that takes bait changes in relation to the number of baits and duration of deployment.

As badger social group territories generally (but not always) contain one main sett and a number of outliers, it is important to consider the question of where vaccine baits should be deployed. Main setts are usually in constant use throughout the year and are the focus of breeding and social interactions within a group, whereas outliers are only intermittently occupied and are less likely to be used for raising a litter (see Delahay et al. 2008). In the present study, of the total of 138 badger capture events recorded during the two seasons, 25 captures (18%) took place at outlier setts that were within the territories of main setts where bait was fed but were not themselves subject to bait deployment. Only one of these animals had not taken bait, suggesting that deployment of bait at main setts alone may be sufficient to target a relatively high proportion of the badger population. This strategy would have several advantages over deploying baits at all setts, including the relative ease in finding and identifying main setts compared to outliers, fewer potentially wasted baits deployed at infrequently used outliers, less time and effort required to deploy baits and hence reduced costs.

The results from the current study illustrate the value of using RhB as a biomarker in future bait uptake studies. RhB has a number of advantages over other available biomarkers; (1) it is commercially available and relatively cheap to purchase, (2) is easily incorporated into baits, (3) requires only a relatively small dose to produce distinctive marking in animal hair, (4) does not require particularly invasive or destructive sampling, (5) is simple to detect in the whiskers or hairs using fluorescence microscopy, and (6) is relatively safe (Fisher 1999). The major disadvantage of using RhB rather than IPA is that unlike the latter, it cannot provide reliable information on the number of baits an individual has consumed (see Massei et al. 2009). However, the relative cheapness (in terms of both market cost and analysis), availability and ease of application make RhB an ideal candidate for large-scale baiting campaigns,

which would require large quantities of bait and biomarker (Southey et al. 2002).

The high proportion of trapped badgers exhibiting evidence of bait consumption in both spring and summer during the present study suggests that the bait-marking method of bait deployment may be a useful approach for delivering an oral vaccine in the future. Nevertheless, rates of uptake will vary with baits of differing composition and in badger populations with less historical familiarity with peanut-based baits. The results suggest that early summer deployment of bait at main setts alone can be used to target a relatively high proportion of the badger social group, including cubs. Estimates of the number of baits deployed per marked badger give first indications of the level of effort (and therefore costs) that may be required to deliver bait to a given proportion of the badger population. Future work will be required to optimise the deployment strategy so as to reduce bait wastage (and hence costs) but retain delivery to a sufficiently high proportion of the badger population.

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