Vaccination of badgers and the control of TB

Submitted by:

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I certify that all materials in this thesis that are not my own work have been identified and that no material has previously been submitted and approved for the award of a degree by this or any other university.

Signature:……

General Abstract

Wildlife diseases can threaten biodiversity, infect humans and domestic animals, and cause significant economic losses. In the UK, bovine tuberculosis persistently affects cattle; some disease control methods target the European badger which acts as a reservoir for the disease. Administering Bacillus Calmette-Guérin vaccine to badgers is a potential approach to reducing the prevalence of the disease across species.

The aim of my studies is to establish whether vaccination, and the potential reduction in disease load, is associated with changes to badger life-history and behaviour. Further investigation is directed at understanding observed variation in bait uptake and badger behaviour towards deployed baits. Analysis investigates whether behaviour or uptake are associated with land-type classes within territories.
The findings I present reveal no significant associations between vaccination and the life-history traits investigated. Both bait uptake rate and behaviour towards bait are shown to have significant associations with the proximity and area of broadleaf woodland/pasture or arable land in the territory. An interesting association showed decreased neophobic behaviour at setts in closer proximity to human habitation. Vaccinated individuals were found to produce significantly lower quantities of gamma-interferon after infection. These additional findings and their relevance to wildlife disease management are discussed.

The results presented give reassuring evidence that vaccination is unlikely to give undesirable side effects; whilst improving understanding of the factors that may affect oral bait vaccination campaigns. Further studies are vital to establish whether vaccination campaigns will reduce the negative impact of bovine tuberculosis in terms of economic impact or health impacts on humans and cattle.

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What is wildlife disease?

Disease, in general, is widely regarded as impairment to the normal physiological functioning of all or parts of an organism. Disease can stem from a number of internal sources including pain or dysfunction; here the focus is on external sources such as infectious disease. Infectious diseases are largely caused by two categories of organisms: macro-parasites, which are multi-cellular organisms living in or on the host, including helminths and arthropods; and micro-parasites including viruses, bacteria, fungi and protozoa [1].

Disease can affect wildlife in a multitude of ways. This can range from reductions in growth, fecundity, increased metabolic requirement and potentially causing death either directly or indirectly through, for example, lack of ability to elude/fend off predators [1]. An example of this is seen in mule deer (*Odocoileus hemionus*) which, when infected with Chronic Wasting Disease (CWD), exhibit symptoms including emaciation, dehydration, decreased fear of humans, lethargy and ataxia [2-3]. CWD, in cervids, is fatal and can cause population declines where the disease is at high prevalence [4].

The significance of wildlife diseases

Wildlife diseases can threaten biodiversity, infect humans and domestic animals, and cause significant economic losses; providing incentives to manage wildlife diseases [5]. With ever increasing human expansion and encroachment into wildlife habitats, comes a growing recognition of the potential importance of wild mammals in the epidemiology of diseases that impact on global human health, agriculture and biodiversity [1]. An example of health threats to wildlife is amphibian chytridiomycosis. This infectious disease caused by the fungus, *Batrachochytrium dendrobatidis*, might be accountable for the potential disappearance of Darwin's frogs [6].

Wildlife sources account for around 72% of all emerging human infectious diseases (e.g. HIV and severe acute respiratory virus), with 335 emergence 'events' between 1940 and 2004 [7]. Despite this, our understanding of wildlife pathogens is relatively poor [1]. These diseases also afflict domestic animals and livestock; causing economic losses from mortality, farm-wide culling and trading restrictions [5]. Examples of this include classical swine fever, foot-and-mouth disease and bovine brucellosis [8-10]. Further impacts of wildlife diseases include domesticated indigenous species, such as deer (*Odocoileus* spp., *Cervus* spp.), which are at risk of infection from free-ranging animals [11].

Ebola-virus-disease is an extremely infectious wildlife disease and a significant zoonosis, well known due to its virulent nature and high mortality rate in humans. The most recent out-break in West Africa has been on-going since March 2014. As of 5th September 2014, 3944 cases and 1759 deaths have been reported [12]. This disease and these out-breaks are not only cause for concern for humans, but also of

great concern for wildlife conservation. Ebola is responsible for a number of devastating epidemics on wild primates [13]. During an out-break in Gabon and Congo over 2002/03, Bermejo *et al.* [14] determined the virus killed approximately 5500 eastern gorillas, a decline of about 83% in the affected region.

Wildlife disease management

Wildlife disease management aims to evaluate, reduce or eliminate the detrimental consequences of wildlife diseases. In a review by Wobeser [11], four basic management tactics are outlined, namely: prevention, control, eradication and *laissez-faire* (doing nothing).

The fact that most wildlife diseases are not managed implies that managers weigh the costs of various actions against the risks of inaction [15]. Delahay *et al*. [1] outline the following reasons why management may usually be carried out:

1. *Direct negative impacts on the host species, such as mortality, population reduction, animal suffering and threats to species survival*
2. *Impacts on ecosystems and the environment, such as infection risks for other wildlife species (through spillover infection), disruption to ecosystems, and impacts on environmental stability and sustainability*
3. *Impacts of disease on domesticated species, including companion, zoo and farm animals.*
4. *Risks to human health if the disease is zoonotic.*
5. *The resource costs of prevention and control of disease in wildlife (including monitoring and surveillance).*

When doing nothing is an unsuitable option, prevention is usually considered the

most practical, economic approach [16].

The European Union imposes strict import controls for animals and animal products; these are in place to mitigate the risks of contagious animal diseases [17]. After an introduction of African swine fever into Spain, Arias & Sanchez-Vizcaino [18] estimated the cost of eradicating the disease to around US $92 million over 5 years, without considering the additional economic impact on pork production and trade. Despite stringent prevention programs; the unsanctioned importing of meat products, either inadvertently by tourists or intentionally by smuggling, presents a continuous threat [19].

Rabies, one of the oldest documented zoonotic diseases, is believed to be infectious to all mammal species, which has enabled it to become rooted in its enzootic environment (animal hosts). Antarctica and Australasia are the only rabies-free continents, although a number of rabies-free countries do exist [20]. Despite the persistence of the disease, oral vaccination programs carried out over 24 European countries, to eradicate red fox (*Vulpes vulpes*) rabies, have been successful in eliminating fox-mediated rabies from large parts of Western and Central Europe [21].

Bovine tuberculosis

A key example of a zoonotic wildlife disease, and the required management regime, is bovine tuberculosis (bTB). *Mycobacterium bovis* is the causative agent of bTB, which infects a wide range of mammalian species including humans. In the UK the disease is being increasingly diagnosed in domestic species other than cattle; increasing the potential sources of infection for cattle, wildlife and humans [22].

In the UK, during the 1930s, approximately 2500 people died annually from bTB, prompting measures to eradicate the public health threat [23]. The majority of measures against bTB are focused around preventing cattle-to-cattle transmission including routine testing and surveillance, pre-movement testing, movement restrictions and the rapid slaughter of infected animals [24]. Cattle bTB testing in 2007 documented 4172 herd breakdowns in England and Wales [25], totalling an annual cost to the taxpayer of almost £100 million; including the costs of compensation for cattle culled, disease research and bTB monitoring. Despite these control methods, the prevalence of bTB in Britain is expected to remain high or even increase. The UK government is reviewing its options for tackling bTB; including badger culling and statutory pre- and post-movement testing of cattle [26].

Badgers: their role in the persistence bovine tuberculosis

The requirement for more effective measures for bTB eradication has led to research and assessment into the European badger's (*Meles meles*) contribution to cattle bTB. Badgers act as a wildlife reservoir, hindering control efforts by transmitting the disease to cattle [27,28]. Direct badger-to-cattle transmission only accounts for an estimated 5.7% of confirmed cattle TB incidence; however, onward cattle-to-cattle transmission amplifies this, accounting for over 54% of incidence in areas with high TB prevalence [28].

Proposed methods for reducing badger-to-cattle transmission

1. Badger Culling

Culling badgers in an attempt to reduce the population, and ultimately disease load, has been proposed and researched fairly extensively (e.g. the Randomised Badger Culling Trial (RBCT) [29]. The results of the RBCT revealed an increase in badger-to-badger disease transmission as a result of culling. The increased transmission observed has been shown to arise from increased movement of individual badgers and the increase in inter-group movements was associated with greater incidences of *M. bovis* infection [30]. Furthermore, culling is opposed by many members of the UK public.

2. Biosecurity

Improving farm biosecurity, using physical barriers to reduce contact between cattle and badgers, has shown 100% effectiveness in preventing badger entry into farm buildings. Whether this will translate into a decrease in cattle bTB incidence is yet unknown [31]. This technique may well be an effective part of a multitude of bTB reduction methods, however it is limited to only preventing contact to cattle and feed within secure farm premises. Disease transmission to cattle and cattle feed outside of farms e.g. pasture and arable land, will likely require addressing.

3. Intra-muscular BCG vaccination

Administering Bacillus Calmette-Guérin (BCG) vaccine to badgers is another proposed method for reducing the prevalence of the disease in the population and potentially reducing disease transfer from badgers to cattle [32-34]. Administering intra-muscular BCG vaccination to captive and free-living badgers has been shown to reduce the progression and severity of *M. bovis* infection after experimental

challenge. It also has been shown to deliver significant reductions to seropositive incidence in vaccinated free-living badgers. Further benefits are shown through reduced likelihood of unvaccinated cubs being seropositive when over a third of their social group had been vaccinated [35-37].

Chapter 2 in this thesis aims to further understand the effects of intra-muscular BCG vaccination programmes on free-living badger populations. Although the ultimate purpose behind vaccination is to reduce/eliminate the disease incidence in cattle and humans, the impact of control measures on the host species' population dynamics and life history traits must be evaluated [38]. This consideration must be made as changes brought about may detrimentally affect disease transmission dynamics and epidemiology [39]. Hence, this chapter initially aims to evaluate any change to the focal life-history traits, that are directly or indirectly associated with BCG administration.

A secondary aim is to conduct a preliminary investigation into the post-infection magnitude of gamma-interferon (IFN γ) responses in badgers. Past studies have shown that the magnitude of the IFN γ response to the ESAT-6 antigen has strong positive correlations with bacterial load and also with disease-associated pathology (in mice [40]; in cattle [41,42]; and badgers [43]). In humans who have recently been exposed to *Mycobacterium tuberculosis,* the levels of IFN γ production in response to the antigen CFP-10 has been identified as an indicator of individuals at a higher risk of developing active disease [44]. These immune responses may underpin any observed alterations to life-history traits between vaccinated and control badgers.

4. Oral BCG Vaccination

An alternative to intra-muscular vaccination is an oral vaccine, which may potentially be a more cost-effective and sustainable method for controlling the disease in badgers [33,45]. Preliminary results in captive badgers, shows that oral vaccination in lipid formulations generates a protective effect [36,46]. The baits and deployment strategy used for oral vaccination must be tailored to the target species, its diet and its ecology. This structured development was the key to the success of the wide-scale vaccination programmes across continental Europe [47], underpinned by a potent and stable vaccine which could be delivered safely and effectively to a single target species (red fox) via the oral route.

In light of this, current field/laboratory studies have established a candidate bait that has the potential to include the vaccine, whilst being attractive to badgers. However, precise details of the formulation of the candidate vaccine baits used cannot be provided as this information is commercially sensitive and data arising from this work will form part of the scientific evidence submitted to the Veterinary Medicines Directorate for any future licence application.

Chambers *et al*. [48] reviews the remaining research requirements that need to be addressed before an oral bTB vaccine can be implemented effectively to badgers. The safety of the oral bait vaccines to non-target species needs to be assessed prior to licensing [49]. Additionally, in order to ensure the cost is viable, the number of baits deployed and the length of deployment needs to be evaluated. Deployment costs may be reduced by pre-baiting for a number of days with bait that does not contain the vaccine formulation [48], potentially increasing vaccine uptake by more 'neophobic' (the tendency of an animal to avoid or retreat from unfamiliar objects or situations) badgers [33]. This needs to be evaluated fully in order to maximise the benefit to the cost.

The ongoing research suggests that the percentage uptake of an oral bait may vary among social groups. This is significant as the effectiveness of an oral vaccination campaign is dependent on the proportion of susceptible individuals that receive the vaccine [33]. As such the third chapter in this thesis aims to provide further understanding into bait uptake variation.

The specific aim of the study is to investigate variation in bait uptake (number of individuals that have consumed the bait) and bait disappearance (the number of baits taken per sett, per day). The behaviour of badgers toward the bait will also be evaluated by investigation into whether badgers exhibit neophobic behaviour towards the bait and bait deployment methods. The composition of the local area land types will also be incorporated into the analysis to see if they have any discernable influence on variation.

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Chapter 2: Bacillus Calmette-Guérin vaccination: its associations with life-history traits and gamma-interferon response in the European badger

Bacillus Calmette-Guérin vaccination: its associations with life-history traits and gamma-interferon response in the European badger

Abstract

Bovine tuberculosis (bTB) persistently affects cattle in Britain and Ireland; some control methods target the European badger (*Meles meles*), which acts as a reservoir for the disease. Previous findings from a four-year clinical field study show that Bacillus Calmette-Guérin administration reduces the chance of vaccinated free-living badgers, and unvaccinated cubs from vaccinated social groups, of testing positive to diagnostic tests. Other control measures have led to unexpected side-effects such as perturbation and an increase in disease transmission.

Using data from the same four-year trial; this study aims to establish whether vaccination is also associated with any changes to badger life-history or behavioural traits. Further analyses investigated whether vaccination alters post-infection gamma-interferon (IFN γ) production.

The findings show no significant associations between vaccination and the life-history traits investigated. However, vaccinated badgers exhibited significantly lower IFN γ production post-infection. Vaccination of wild badgers is a proposed control method for reducing bTB incidence in cattle; the results of this study give reassuring evidence that negligible undesirable side-effects may ensue. Previous studies have shown that post-infection IFN γ magnitude can predict the likelihood of active disease progression. Here BCG vaccination is shown to associate with lower production, providing further understanding the underpinnings of BCG's prophylactic effect.

Keywords: European badger; Tuberculosis; Bacillus Calmette-Guérin; Life-history traits; Gamma-interferon.

1. Introduction

Mycobacterium bovis causes chronic infection (Bovine tuberculosis, bTB) in a wide range of mammalian hosts, persistently affecting cattle in large parts of Great Britain and Ireland [1,2]. Disease persistence has been linked, in part, to the European badger (*Meles meles*); which acts as a reservoir for the disease [3]. A gamma-interferon (IFN γ) release assay (IGRA) using specific antigens ESAT-6 and CFP-10, which works by stimulating lymphocytes [4], is able to detect relatively early stages of infection in badgers [5]. ESAT-6 and CFP-10 antigens are used because vaccination with BCG can compromise the specificity of tuberculin-based assays [5]. Whilst detection of those more likely to be at advanced stages of *M. bovis* infection is possible though a lateral-flow immunoassay (BrockTB Stat-Pak; Chembio Diagnostic Systems, New York, USA) to detects serological responses or bacterial culture which identifies active excretion of *M. bovis* [6].

One proposed bTB control measure is to administer Bacillus Calmette-Guérin (BCG) vaccine to badgers [7-9]. In captive badgers, intramuscular BCG vaccination has been shown to confer a degree of protection through a reduction in the progression and severity of *M. bovis* infection after experimental challenge [10,11]. BCG vaccination also resulted in a 73.8% reduction of seropositive incidence in free-living badgers [12]. Further benefits were observed in unvaccinated cubs. When over a third of their social group had been vaccinated, the likelihood of being diagnosed as seropositive, to a combination of tests, was reduced by 79% [12]. Vaccination of wildlife has also shown some success in disease control; for example, oral vaccination of wild boar (*Sus scrofa sp*.) against classical swine fever has maintained study areas with high levels of immunity and low viral incidence [13].

Vaccination of red foxes (*Vulpes vulpes)* has also proven to be effective; large numbers of European countries have consequently eliminated all cases of rabies [14].

The ultimate purpose behind vaccination of badgers is to reduce/eliminate the disease incidence in cattle and humans. It is, however, also necessary to understand how disease control measures will impact the targeted species' population dynamics and life histories [15]; as these in turn may affect disease transmission dynamics and epidemiology [16]. For example, in badgers, culling is associated with increased movement; and inter-group movements are associated with greater incidence of *M. bovis* infection [17].

The aim of this paper is to evaluate any changes to life-history traits that might be associated with BCG administration and the consequent reduction in bTB incidence. bTB infection is associated with changes to individual and population level life-history traits and behaviours in various species. In badgers, both sexes exhibit declining body condition with progressing levels of infection [18], with significantly lower survival rates observed once an individual reaches the point of excreting or shedding bTB bacilli [19]. Additionally, female reproduction has been shown to positively correlate to the animal's body condition [20]. bTB also affects fecundity and mortality rates in African buffalo (*Syncerus caffer*), reducing pregnancy rates in infected females of most age groups by 27% [21] and increasing annual mortality risk by 11% [22].

Badgers infected with bTB forage further into neighbouring territories [23], spending significantly more time interacting and residing amongst foreign social groups compared to uninfected individuals [24]. Vicente *et al.* [25] discuss how bTB

incidence is associated with groups with higher rates of movement. Similarly, tuberculous brushtail possums (*Trichosurus vulpecula*) travel larger distances than uninfected individuals [26]. In light of this, it is clear that bTB does have an effect on life history traits in badgers and other species. The analyses performed here therefore aim to distinguish whether vaccination reduces the adverse impacts that the disease has on badger life-history traits.

The final aim is a preliminary investigation into whether there are significant, detectable differences in the post-infection magnitude of IFN γ response, which may underpin any observed alterations to life-history traits between vaccinated and control badgers. Past studies have shown that the magnitude of post-infection IFN γ response to the ESAT-6 antigen has strong positive correlations with bacterial load and also with disease-associated pathology (in mice [27], in cattle [5,28] and recently in Badgers [29]). In humans recently exposed to *Mycobacterium tuberculosis* the levels of IFN γ production in response to CFP-10 can be used as an indicator to identify individuals at higher risk of developing active disease [30]. Furthermore, IFN γ production may offer a biomarker for better disease prognosis, which would be a promising step towards a highly desirable tool for more efficient tuberculosis control [30].

2. Materials & Methods

This was an opportunistic study, utilising data collected from a four-year parallel field-study. For the full methodology on study area and population, as well as badger trapping, sampling and vaccination methodologies see Chambers *et al.* [10] and Carter *et al.* [12]. A shortened reiteration is provided below for reference.

Field work was carried out in an area of mixed woodland and agricultural land covering approximately 55 km^2 in Gloucestershire, southwest England, between November 2005 and October 2009. The area was chosen as it is within a region of moderate to high badger density, where bTB is known to be endemic in the badger population and where there had not been recent badger culling. Badger social groups were identified by sett surveys and bait marking and allocated to "vaccinate" or "control" treatment following a baseline trapping session in summer. Treatments were allocated at a ratio of 60:40 (vaccinate:control) Once a social group had been allocated as a vaccinate group, all animals first captured in that group were vaccinated irrespective of their *M*. *bovis* test status. Animals originating from vaccinate groups but caught in subsequent years in a control group were repeatedly vaccinated according to their original treatment allocation.

Badgers were captured in steel mesh traps baited with peanuts and set for two consecutive nights following a three to ten day pre-baiting period. All active setts in the study area were trapped at least twice a year, other than in 2007 when a foot and mouth disease outbreak prevented the second trapping operation from taking place. Upon first capture each animal was marked with an identifier microchip and a tattoo on the abdomen with the corresponding unique three-digit identification number. For

each capture event, the trap location, sex and age were recorded. Clinical samples were taken from all badgers at each capture event, where possible. BCG Danish strain 1331 vaccine (Statens Serum Institut (SSI), Copenhagen, Denmark) was supplied at 2 - 8 x 10^6 colony-forming units (CFU) per vial. The vaccine was prepared by adding 1 ml of Sauton diluent to each vial. BCG vaccine was administered on recapture at a rate of one dose per calendar year, resulting in some badgers receiving multiple vaccinations over the course of the study. After sampling and treatment, captured badgers were returned to their point of capture and released.

Diagnostics

Diagnostic testing was also carried out following the same methodology as Chambers *et al.* [10] and Carter *et al.* [12]; this is partially reiterated with some revisions: Bacterial culture, used to identify active excretion of *M. bovis*; and the Brock (TB) Stat-Pak test, which detects serological responses, were used as evidence of more progressed infection [6]. The IGRA using *M. bovis* specific antigens (ESAT-6 & CFP-10P) was used because the 'traditional' test using Purified Protein Derivative-Avian (PPD-A) & Purified Protein Derivative-Bovine (PPD-B) antigens may be compromised by BCG vaccination leading to reduced specificity [5].

Statistical analyses

Mixed models were used as they allow for the inclusion of random effects, which is essential for studies of free-living populations which exhibit natural variation among individuals, different groups, and over time [31]. In all mixed models; social group

was included as a random factor to account for this variation, individual ID was also included when multiple data points existed for an individual. Group level analyses were done using data compiled separately for each of the co-existent social groups in a given year; groups were assigned annually based on bait-marking data (see Chambers *et al.* [10] and Carter *et al.* [12]).

Two statistical programs were used for the analysis. 'SPSS' 20.0 (SPSS Inc., Chicago, IL, U.S.A.) was used for performing linear mixed models, fitted with restricted maximum likelihood, (REML LMM) on continuous response variables. 'SPSS' was also used to perform a general linear model (GLM) for the IFN γ related analysis. The 'lme4' package [32] for version 2.15.2 of the 'R' statistical program (R Development Core Team 2012) was used for performing generalised linear mixed models (GLMM), fitted with the Laplace approximation, on binary/Poisson response variables.

Random effects were retained in all models to avoid 'sacrificial pseudo-replication' [33]. Table 1 outlines the full covariate, fixed effect and random effect inclusions for each of the models performed. Step-wise removal of least significant, non-significant terms was used to obtain final model formulas.

At each capture event, badger age was classified as either 'cub' or 'adult'. Badgers first captured as cubs were cubs only for the calendar year. For cub trait analyses 'age' was measured as the time, in days, since February 15th of the year. This is to coincide with the time of highest births; which usually falls between mid‐January and mid‐March, peaking during the first fortnight in February [34].

Infection status at each capture was recorded using a one-way progressive system, akin to that described by Delahay *et al.* [7] and Tomlinson *et al.* [18]. Infection

statuses were graded ‘0’, ‘1’ or ‘2’: Badgers with no previous/current positive results to any diagnostic tests used were considered ‘negative’ (‘0’). A current/previous positive IGRA test was considered a representation of early disease (‘1’). A current/previous positive result to either bacterial culture or the Brock (TB) Stat-Pak test was considered to be evidence of more progressed infection (‘2’). Once at a higher level no regression to a lower state was possible. Initial infection statuses (disease status at first capture) were used instead of an individual/group’s current status. This was done to avoid concealing any effects that vaccination’s reduction of disease incidence/progression may generate or supress. A group’s status was assigned based on the presence (y/n) of individuals with progressed disease - for the group reproductivity analysis; this was replaced with ‘reproductive females with progressed infection’ as they have most contact with the cubs, before their first emergence.

Reproductive status of a female was based on presence/absence of signs of previous or current lactation – based on teat examination (see Dugdale *et al.* [35]). Group reproductivity was based on the number of cubs trapped in a social group per calendar year.

To investigate whether the effects of vaccination are compounded over the course of the study (i.e. does the proportion of the population vaccinated increase and disease incidence reduce with each study year, leading to increasing effect of treatment?). This was accounted for by the inclusion of an interaction between study year and treatment in each of the mixed models; but removed first if not shown to be significant.

IFN γ response

Table 1 shows the models fitted and the terms included. The response variable was the individual's response to CFP-10 & ESAT-6 antigens minus their response to purified protein derivative–Avian (PPD-A) which accounts for an individual's exposure to *M. avium* or other cross-reactive environmental mycobacterial antigens [36]. The interaction between treatment and days since previous capture was included as a proxy for time since vaccination (where applicable); in case responses were affected by time since vaccination. Only individuals known to be previously negative to all diagnostic tests (and vaccinated, where applicable), then becoming positive to only the IGRA were used in the analysis. This gives an indication of an individual's IFN γ production as close to the infection point as possible, allowing comparison of vaccination and control individual's responses. An addition of 1 was applied to all data-points to ensure all values were positive. Values were then log-transformed to satisfy the conditions of normality required by the analysis.

Table 1. **Summary of models fitted to investigate effect of vaccination on badger life history and the variables included to analyse each response variable**

model name	statistical analysis (family)	response variable	explanatory variables		
			fixed	random	interactions
adult body weight	LMM (Gaussian)	weight (kg)	sex, body length, month, year, breeding status, initial infection status, and treatment	individual and social group	treatment: year
cub growth	LMM (Gaussian)	increase per day in weight (g) or length (cm)	sex, initial capture body length & weight, year, initial infection status, and treatment	social group	treatment: year
social group size	GLMM (Poisson)	individuals trapped in social group	year, initial presence of individuals with 'progressed' infection, and treatment	social group	treatment: year
group reproductivity	GLMM (Poisson)	new cubs captured in a calendar year	year, number of reproductively active females, initial presence of reproductively active females with 'progressed' infection and treatment	social group	treatment: year
female reproductive status	GLMM (binomial)	signs of previously/current lactation	year, season (summer/autumn), initial infection status, and treatment	individual and social group	treatment: year
cub survival	GLMM (binomial)	captured as adult (y/n)	birth year, age at first & last cub capture, initial infection status, and treatment	social group	treatment: birth year and treatment: first cub capture
inter-group movement	GLMM (binomial)	trapped in different social group in the following capture	sex, days since previous capture, previous capture year, month, initial infection status and treatment	individual	treatment: previous capture year
IFN *γ* response	GLM (Gaussian)	(CFP10 & ESAT6) – (PPD-A) response: at first positive result	sex, age, days since previous capture, and treatment	social group	treatment: days since previous capture

3. RESULTS

Over the period of the study a total of 842 (522 vaccinated, 320 control; 394 male, 448 female) individual badgers were trapped, with a combined 1783 (1163 vaccinated, 620 control) captures between them. Number of captures ranged from 1 to 8 per animal. 438 of the badgers caught were caught as cubs (281 vaccinated; 158 control).

Life-history traits

Treatment with BCG vaccine did not significantly affect any of the life history traits investigated with no consistent directional trend of treatment (Table 2). Initial infection status was significant in a number of the analyses. Consistent with other studies; badgers with evidence of more progressed infection showed lower adult body weight, the least chance of being a reproductively active female ($P<0.05$), and cubs with evidence of more progressed infection were considerably less likely to be re-captured as an adult. At a group level; groups that initially included individuals with evidence of more progressed infection correlated with larger group sizes.

IFN γ response

Given the restrictions outlined in the methods section, only 30 individuals (14 vaccinated; 16 control) were suitable for inclusion in the analysis. The only significant explanatory variable that remained in the reduced model was treatment. Vaccinated individuals were shown to be significantly associated with considerably lower *IFN γ*

production (see Table. 2 & Figure. 1) than control individuals. Control individuals on average produced *IFN* γ optical density values of 0.40 (at 450nm) (before the increase of 1.00 and log-transformation to normalise data), vaccinated individuals only produced 0.07 (17.5% of that seen in control individuals) in comparison.

Table 2. **Summary of the significant terms for each of the reduced models**

response variable	parameter	statistic	P-value	estimate
adult body weight	treatment	$F^{Control}_{1,1162} = -1.24$	0.215	-0.09
	sex	$F^{Female}_{1,1162} = -4.55$	<0.001	-0.41
	breeding Status	$F^{Active}_{1,1162} = 3.40$	0.001	0.32
	month*	$t^{June}_{4,1162} = -14.58$	<0.001	-1.48
		$t^{July}_{4,1162} = -11.60$	<0.001	-1.32
		$t^{October}_{4,1162} = 9.75$	<0.001	1.10
	* = against September	$t^{November}_{4,1162} = 5.47$	<0.001	1.68
	year*	$t^{2006}_{3,1162} = 1.16$	0.103	0.15
		$t^{2007}_{3,1162} = 4.58$	<0.001	0.51
	* = against 2009	$t^{2008}_{3,1162} = 5.74$	<0.001	0.55
	body length	$F_{1,1162} = 25.69$	<0.001	0.03
	initial infection status*	$t^{0}_{2,1162} = 1.53$	0.128	0.15
	* = against status "2"	$t^{1}_{2,1162} = 2.68$	0.007	0.39
cub growth - length	treatment	$F^{Control}_{1,92} = 0.57$	0.571	0.06
	year*	$t^{2006}_{2,92} = -0.94$	0.003	0.67
	* = against 2009	$t^{2008}_{2,92} = -3.32$	0.246	0.15
	initial body length	$F_{1,92} = 2.30$	0.023	-0.36
	initial body weight	$F_{1,92} = -5.89$	<0.001	<0.01
- weight	treatment	$F^{Control}_{1,91} = 0.96$	0.327	3.76
	initial body weight	$F_{1,91} = -6.09$	<0.001	-13.86
	initial body length	$F_{1,91} = 2.30$	0.001	-0.36
	year*	$t^{2006}_{2,92} = 6.89$	<0.001	67.70
	* = against 2009	$t^{2008}_{2,91} = 1.56$	0.123	16.88
	month*	$t^{October}_{2,91} = 4.12$	<0.001	22.77
	* = against September	$t^{November}_{2,91} = 4.07$	<0.001	61.15
social group size	treatment	$z^{Vaccine}_{1,106} = 1.36$	0.163	0.18
	initial presence of individual(s) with progressed infection	$z^{Yes}_{1,106} = 4,20$	<0.001	0.38
group reproductivity	treatment	$z^{Vaccine}_{1,202} = -0.20$	0.842	-0.03
	number of reproductive females	$z_{1,202} = 8.69$	<0.001	0.49
female reproductive status	treatment	$z^{Vaccine}_{1,627} = -1.14$	0.256	-0.35
	initial infection status*	$z^{1}_{2,627} = 3.58$	<0.001	2.05
	* = against Status ''0''	$z^{2}_{2,627} = -0.11$	0.913	-0.05
	year*	$z^{2007}_{3,627} = 2.81$	0.005	0.94
		$z^{2008}_{3,627} = -0.52$	0.602	-0.17
	* = against 2006	$z^{2009}_{3,627} = 3.36$	<0.001	1.06
cub survival	treatment	$z^{Vaccine}_{1,400} = -0.41$	0.681	-0.15
	initially infected*	$z^{1}_{2,400} = -0.93$	0.352	-0.48
	*= against status ''0''	$z^{2}_{2,400} = -2.38$	0.017	-1.28
	year*	$z^{2007}_{3,400} = -7.45$	<0.001	-3.01
		$z^{2008}_{3,400} = -3.43$	0.001	-1.47
	* = against 2006	$z^{2009}_{3,400} = -8.06$	<0.001	-5.29
inter-group movement	treatment	$z^{Vaccine}_{1,938} = -0.50$	0.620	-0.68
	days since previous capture	$z_{1,938} = 2.62$	0.009	<0.01
IFNγ response	treatment	$t^{Control}_{1,28} = 2.74$	0.011	0.23

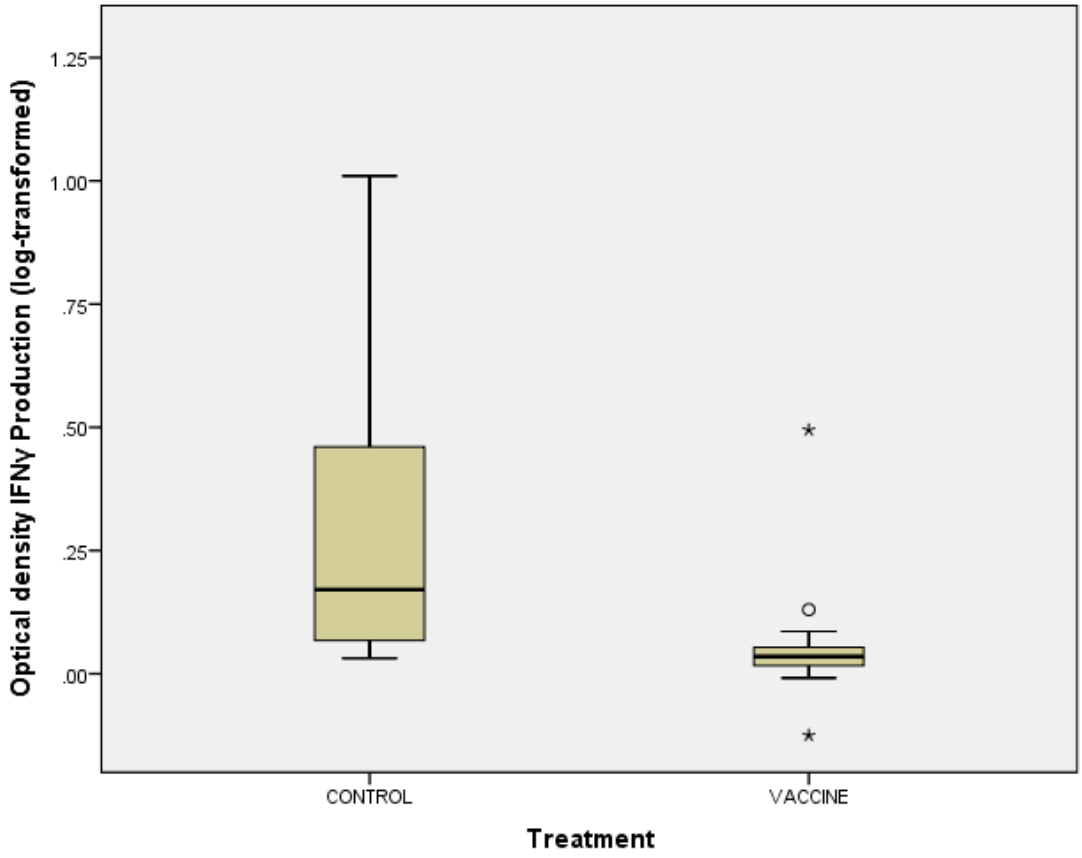

Figure 1.

Box-plot representing log-transformed values of post-infection gamma-interferon production; of control and BCG-vaccinated badgers, respectively.

4. Discussion

The principal aim of this study was to determine vaccination's ancillary effects on life history traits of the European badger. To date, I am unaware of any studies that have investigated the effects of vaccination, as a disease control method, on the ecology of the species vaccinated. It was hypothesised that due to the deleterious effects of bTB infection; vaccinated individuals and treatment groups might be less afflicted by the detriment of infection. However, all trends were non-significant with no consistent directional trends for treatment's effect. Annual and monthly variations were significantly associated with cub production, growth rate and survival rates. This is consistent with previous understanding that the majority of variation in these traits is explained by differing food availability between years and between territories [37].

The findings are in line with Tomlinson *et al.*'s [18] findings, which show a negative correlation between body condition and infection in adult badgers. However, the analyses here did reveal an anomalous result; as expected individuals starting with progressed infection had the lowest body weight, but, individuals with signs of early disease were associated with significantly higher body weights than uninfected adults. A potential explanation for this is that individuals are more likely to become infected if they have wider ranging territories, with more interaction to other social groups/individuals, however the benefit is an increased foraging area and better access to food resources. Infection status could also have been anticipated to be associated with inter-group movement, however, this was not found to be significant.

As hypothesised IFN γ production did show an association with treatment; vaccination was associated with significantly lower production of IFN γ (Figure 1). This is consistent with previous studies [5,26,28-29] showing that vaccination of badgers is associated with significantly decreased incidence and progression of bTB and with lower IFN γ production. Unfortunately, the available sample size and experimental design here did not allow for future disease progression do be included in the analysis. Future studies could examine whether a predictable link does exist between IFN γ production and disease progression in badgers. This could be valuable as IGRAs might enable the identification of asymptomatic individuals who, based on their magnitude of IFN γ response, are likely to develop active bTB [30,38]. If realistic, this could be a beneficial tool towards tuberculosis management in humans, badgers and other wildlife.

In conclusion, no significant associations were made between BCG vaccination and changes to the life-history traits of badgers. Although not the predicted outcome of the study, it can still be viewed as encouraging from the disease management view point. Encouraging in the sense that vaccination does significantly lower disease incidence [10,12], but without any accompanying changes to the traits/behaviours examined here. For instance if a vaccination regime were to lead to increased survival or productivity; the potential increase in host density could reduce the benefits of vaccination.

Additionally, the results of a preliminary investigation into IFN γ production are consistent with those in previous studies. Thus, continuing confirmation that IGRAs have the potential to provide more than just binary prognosis of infected/not infected based on a cut-off value.

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Chapter 3: Investigating the effect of social group territory characteristics on the consumption of a placebo vaccine bait, targeting the European badger

Investigating the effect of social group territory characteristics on the consumption of a placebo vaccine bait, targeting the European badger, *Meles meles*

Abstract

Bovine tuberculosis is a serious cattle health concern in the UK. The European badger hinders control efforts through transmission of the disease from badgers to cattle. In a bid to reduce the transmission between badgers and cattle, an oral vaccine bait is being developed to administer Bacillus Calmette-Guérin vaccine. Current studies demonstrate varying levels of bait uptake between different badger social groups and individuals.

Social group territories are comprised of different land types, representing varying diets and available food sources. Social groups will also have differing access and familiarisation to human habitation and access routes. The aim of this study is to investigate the variation in bait uptake (number of individuals that have consumed the bait), bait disappearance (the number of baits taken per sett, per day) and badger's behaviour toward bait deployment that might be associated with the characteristics of the local area.

The proximity and area of broadleaf woodland, arable land and pasture in a territory were important factors for each of the response variables studied. An interesting association showed decreased neophobic behaviour at setts in closer proximity to human habitation. The findings are potentially valuable in the development of bait deployment methods. The importance of the focal species' ecology in the development of targeted baits is also discussed.

Keywords: oral vaccine; bovine tuberculosis; *Meles meles;* habitat effects.

1. Introduction

Bovine tuberculosis (bTB), a significant zoonosis, is a serious animal health concern in the UK, the associated cost inflicted onto the taxpayer and farming industry is unsustainable [1]. Cattle bTB testing and consequential slaughter, the primary method for disease eradication, is proving ineffective, not helped by diagnostic testing that lacks the sensitivity to efficiently diagnose all infected cattle [2]. Furthermore, although cattle-to-cattle transmission is an important factor in the spread of disease [3]; the European badger (*Meles meles*) acts as a wildlife reservoir, hindering control efforts by transmitting the disease to cattle [4,5]. Donnelly & Nouvellet [5] estimate that in areas of high cattle bTB incidence, over 54% of the total cases stemmed from the original badger transmissions. Implementation of effective measures to reduce the transmission risk of this disease between badgers and cattle are urgently required.

Studies, including the randomised badger culling trial [6] and current culling pilot studies, have focused on establishing the efficacy of badger culling as a potential disease control measure [7,8]. A number of studies (e.g. [7,9-10]) have analysed the number of incidents of bTB in cattle after the end of a culling experiment, the overall conclusion is that for culling to be effective it would have to be persistent and of a large scale (and costly in terms of both public opinion and money).

Another potential control measure is to improve farm biosecurity i.e. physical barriers to reduce contact between cattle and badgers. This approach has shown that simple exclusion measures were 100% effective in preventing badger entry into farm buildings. It is not yet known how this translates into reducing disease transmission [11]. Although simple methods such as this may be beneficial to controlling bTB,

exclusion may not always be practical and limiting contact may prove impossible in certain areas e.g. pastures, so other strategies need to be considered.

Vaccinating badgers against bTB is a potential strategy to reduce the spread between badgers and cattle without the animal rights concerns that come with culling. Intramuscular Bacillus Calmette-Guérin (BCG) vaccination of captive badgers confers a degree of protection, reducing the progression and severity of *Mycobacterium bovis* after experimental challenge [12,13]. Further benefits were observed in free-living badgers as a study by Carter *et al*. [14] showed; intramuscular injection of BCG reduced, by 76%, the likelihood of vaccinated individuals testing positive to a combination of diagnostic tests of progressive infection. Benefits were also transferred to un-vaccinated cubs; with a 79% reduction in likelihood of a positive result to a similar panel of tests (when a third or more of their social group had been vaccinated).

Cage trapping and vaccinating of badgers is labour intensive, with potentially prohibitive costs. A recent report by the Welsh government published the first year expenses of their vaccination programme, revealing costs of approximately £3275 per km^2 [15]. Alternatively to intramuscular vaccination, an oral vaccine is a potentially more cost-effective and sustainable method [16,17]. Preliminary results show that oral BCG vaccination, in a lipid formulation, generates a protective effect in captive badgers [18,19]. Additionally, oral vaccination could resolve some of the shortcomings of intramuscular vaccination, such as eliminating the need for cage trapping which may show bias towards the capture of only certain individuals and the potentially adverse immune response associated with stress of cage-trapping [20].

Oral vaccination has had its successes in combating disease in wildlife e.g. the vaccination of red foxes (*Vulpes vulpes*) against rabies; deployment across a number of European countries has proven successful in eliminating all cases of the disease [21]. Initiatives against classical swine fever in wild boar (*Sus scrofa sp*.) have maintained low viral incidence and high immunity levels, in the areas studied [22].

To be effective the baits and deployment strategies used for oral vaccination must to be tailored to the target species, its diet and its ecology [23,24]. This is important, as the effectiveness of an oral vaccination programme is dependent on the proportion of susceptible individuals that receive the vaccine [16]. To increase the effectiveness of rabies vaccination campaigns in a number of European countries, Vos *et al*. [24] determined that an annual vaccination campaign in autumn would be the most cost-effective approach; reaching both the adult and juvenile fox population. This was deemed most appropriate way to increase the relatively low vaccination coverage of young foxes, owing to the fact that adult foxes were able to locate the distributed baits more often than cubs during summer campaigns [25,26]. This highlights the requirement of wildlife management programmes being fully researched before implementation.

Using advanced aerial and bait-station deployment methods a study by Tompkins *et al*. [27] achieved a 95–96% efficacy of orally delivered BCG vaccine to the common brushtail possum (*Trichosurus vulpecula*). This efficacy should be more than sufficient for the purpose of eradicating tuberculosis from wild possum populations, giving confidence that oral vaccination is a tenable solution for the control of bTB in wildlife [27]. A number of on-going field/lab studies are developing the best bait and deployment method for oral BCG vaccine delivery to badgers. These studies have

established a candidate bait that has the potential to contain the vaccine, whilst being attractive to badgers. On-going research into the development of an oral bTB vaccine for badgers suggests that percentage uptake of an oral bait may vary among social groups. Further investigation is required to help explain this variation in order to maximise the success of any future deployment.

The proximity and proportion of land types in the surrounding area will represent different diets and varying available food abundance for a social group. The specific aim of the study is to investigate variation in bait uptake (number of individuals that have consumed the bait) and bait disappearance (the number of baits taken per sett, per day). The behaviour of badgers toward the bait will also be evaluated by investigation into whether badgers exhibit neophobic behaviour towards the bait and bait deployment methods. The composition of the local area land types will also be incorporated into the analysis to see if they have any discernable influence on variation. Specifically investigating any variation attributed to the proximity and proportion of local land types, the proximity to human access (e.g. roads, footpaths etc.) and the proximity to human habitation (e.g. homes, farm buildings etc.).

Despite the wide-ranging consequences of neophobia toward novel foods, few studies have investigated the underlying factors determining it and these have usually been in captivity [28]. Caution towards unusual foods helps herbivores survive in a world where the nutritional and toxicological characteristics of food sources are constantly changing [29].

Although badgers specialise on earthworms over much of their range, they are able to exploit a wide variety of other foods [30]. For instance, the availability of human habitation in the territory was found to be significantly related to badger bodyweight,

potentially benefiting from supplementary food e.g. garden fruit, cattle feed, and domestic refuse [31,32]. This variation in surroundings may, therefore, affect tendencies for badgers to require or turn to new resources (i.e. baits). For example, increased familiarisation with anthropogenic food sources or disturbance may make the bait seem less ‘foreign’.

Neophobic behaviour (the tendency of an animal to avoid or retreat from unfamiliar objects or situations) of badgers towards bait deployment will be the focus of the investigation into local area characteristics on behaviour. This type of behaviour has been studied in numerous species. For example in birds of prey, only without familiar food being available will the bird, hesitantly, eat novel prey [33]. Captive wild rats were shown to exhibit large individual variation in the responses to new foods and food containers placed into their home range, with neophobic behaviour toward new food containers being significantly stronger than that shown to new foods [34]. Free-living foxes also show neophobic behaviour, with significantly reduced visitation and bait consumption rates to bait stations after the introduction of a novel object [35]. Despite an abundance of studies presenting examples of neophobia, there does not appear to be any that try to elucidate correlates of this behaviour. This study aims to establish if badgers show this aversive behaviour and if the characteristics of the local area have any discernable influence.

2. Methods and Materials

This study utilises data from an on-going lab and field based study into the development of a bait and deployment method that is best suited for the delivery of an oral BCG vaccine bait to free-living badger populations. Precise details of the formulation of the candidate vaccine baits used cannot be provided as this information is commercially sensitive and data arising from this work will form part of the scientific evidence submitted to the Veterinary Medicines Directorate for any future licence application. However, the statistical analyses have controlled for bait-related variables that may have influenced the uptake and disappearance of deployed baits.

Study areas

The setts used in the study were spread across six counties in the South/South-West of England, all of which are regions in which cattle bTB is highly prevalent [6]. Data were collected in an attempt to gather information from badger populations in a range of areas, similar to those in which oral vaccination is likely to be used. Each year, setts were only selected which had not been subjected to any studies in the past; in an aim to avoid bias in the study through badger populations that had previous exposure to similar bait or feeding methods.

Bait marking, a method for establishing social group territories (see [36]) would involve exposing the setts to factors (e.g. baits and human disturbance) that would potentially influence the results. Without carrying out the bait marking process it was not possible to determine the territory associated to each sett. To combat the

possibility that the social groups of two study setts would overlap; setts were used only if a) they were identified as a main sett and b) that they were at least 2km from another experimental study sett. A study by Rogers *et al*. [37] estimated that the distances moved by badgers between social groups were mostly less than 1000m, with social group main setts (considered as those that are the permanent residence of a social group throughout the year) roughly 600m apart. Therefore, study setts being selected to be at least 2 km apart provides a conservative buffer between setts. For the locations, number of setts used, study periods see Table 1.

Table 1. summary of some variances in experimental design factors between study years

year	locations	number of setts	number of baits	study days	pre-bait	number of badgers
2010: May June, July, August	Gloucestershire, Somerset & Bedfordshire	44	15	12	NA	192
2011 May June, July	Gloucestershire & Devon	38	15	12	NA	316
2012 July, August	West Sussex	25	8 or 16	8 or 12	4 or 8 days	70
2013 August	Gloucestershire & Wiltshire	7	24	10	NA	NA

Experimental design

Baits were deployed in the afternoon to minimise interference from non-target species. Baits were placed to cover the majority of the sett, usually placed just off runs. Apart from in 2012, where baits were deployed directly down holes, baits were deployed under slabs (30cm x 30cm, ≈ 2.5kg) to help assess disappearance and consumption by non-target species. They were deployed with disposable gloves, changed with each new sett, to minimise presence of anthropogenic smells on baits/tiles (where applicable).

For the purpose of the study *bait uptake* refers to the number of individuals that have consumed the bait, this is measured as badgers that are detected to have biomarker (see below), that was mixed into the baits, present in their blood. *Bait disappearance* from here on is the number of baits taken from a sett per day.

In all the years in which bait uptake was monitored, the baits contained one of three different analogues of Iophenoxic Acid (IPA): 1) Propyl IPA (P-IPA); 2) Isobutyl IPA (IB-IPA) and 3) Ethyl IPA (E-IPA). IPA is a traceable biomarker that allows individuals that have ingested bait to be confirmed (see [23]). This was used to establish the bait uptake response variable. For the number of badgers caught within given years see Table 1.

Badger trapping

In the years in which the uptake of biomarker was measured (2010 – 2012), badgers initially needed to be trapped in order to obtain samples to test for the presence of the biomarker. Badgers were trapped in steel mesh traps, after a period of one week's pre-baiting, approximately two weeks and four weeks after the end of the

biomarker feeding. The second trapping session was primarily an attempt to maximise the number of animals captured within each group. All captured badgers were brought to a purpose built mobile sampling facility, centrally located in the study area, or to the facility at AHVLA Woodchester Park, Gloucestershire. Up to two samples of blood (up to 8.5ml, dependent on body weight) were taken from each animal, under anaesthetic, following a similar methodology to that of McLaren *et al*. [38].

Neophobia

Two Bushnell trail cameras (Bushnell Trophy camera model 119435) were placed at each of the seven setts in 2013, directed at active sett areas (holes and runs). Cameras were motion activated, taking 60-second video with 1-second intervals (the minimum) between each clip. Batteries and SD cards were checked daily. The cameras were positioned with a field of view covering a cross-section of the slabs covering the baits. This footage was used to determine a proxy for neophobia i.e. the time the first bait was observed being taken. The time bait was taken was recorded as the number of hours after midday on the day of deployment, as baits were frequently taken during daylight hours the time was from midday rather than sunset. The maximum time of 24 hours was recorded to setts at which no bait was taken on a given night.

Although there were only two cameras used to determine the time the bait was taken, this was consistent across setts. Cameras were also placed to view areas of seemingly high sett activity and with as high a number of baits in view as possible.

The time baits were placed at the sett was included in analyses to account for any bias towards setts at which bait was administered earlier.

Land types

The land types that were of interest were the habitat types: broadleaf woodland, arable land and improved grasslands (pasture, from herein). These types were chosen as not only are they the most abundant land types observed, but they are also of ecological importance to badgers [32].

The proportions of, and distances to the land types were gathered using the Land Cover Map 2007 [39], Land Cover Map 2007 is a parcel-based thematic classification of satellite image data covering the entire United Kingdom. The 'extract by circle' function in ArcMAP 10.1 (ESRI, California) was used to extract the attributes of 300m radiuses around each sett, representing local land type proportions. The 'measure' tool was utilised for establishing the proximity of access routes (e.g. roads, footpaths, bridleways etc.), habituation (human settlements) and the three land types of interest. This was done in order to obtain representative figures for the local area characteristics that were to be included in the analysis.

A limitation of this study was the use of Land Cover Map 2007 data, used to obtain land type data. This data was collected in 2007, and in the time between then and these studies taking place in 2010-2013 there was potential for change to have occurred (estimate unavailable).

Statistical Analyses

During the development of this commercial vaccine delivery bait; cubs have shown a tendency towards higher uptake than adults, to account for this age was included as a fixed factor in appropriate models. Bait type was also included as a fixed factor, as variable uptake rates have been observed with different candidate baits. The number of baits deployed did not improve the AICc model fit for the uptake response variable, showing little predictive value (results not shown) and so was not included in global models.

The statistical programming software 'R' (R Development Core Team, 2009) was used for all analyses and graphical production. The 'lme4' package [40] was used to fit each of the global models. All analyses were performed using generalised linear mixed models (GLMMs). Mixed models allow for the inclusion of random effects, this is necessary for studies of free-living populations that exhibit natural variation between individuals, groups, and over time [41].

Table 2 conveys the full global model inclusions for each of the models, indicating all of the variables included and the error structures fitted to each of the models.

The 'standardize' function in the 'arm' package [42] was used to standardise the predictors. Centralising predictors is essential when model averaging is employed, and standardization facilitates the interpretation of the relative strength of parameter estimates (see [42]). A sub-set of best fitting models were obtained from the global models using the 'dredge' function in 'MuMIn' package [43]. Akaike's Information Criterion information-theoretical (AIC-IT) approach was used for model selection and model averaging [44], all models within 2 **Δ**AICc of the model with best fit were included in the selection and averaging.

Table 2. Summary of model types and the parameters included to analyse each response variable

model name	statistical analysis (family)	response variable	parameters	
			fixed	random
bait disappearance	GLMM (Poisson)	number of baits taken per day	day of study, bait type, total baits put down, distance to habitation, distance to access, distance to broadleaf woodland, distance to arable land, distance to pasture, area of broadleaf woodland within 300m (m^2), area of arable land within 300m (m^2), area of pasture within 300m (m^2)	sett ,year
neophobia	GLMM (Gaussian)	time the first bait taken	day of study, time bait put down, distance to habitation, distance to access, distance to broadleaf woodland, distance to arable land, distance to pasture, area of broadleaf woodland within 300m (m^2), area of arable land within 300m (m^2), area of pasture within 300m (m^2)	sett
bait uptake	GLMM (binomial)	biomarker ingested	age, sex, bait type, distance to habitation, distance to access, distance to broadleaf woodland, distance to arable land, distance to pasture, area of broadleaf woodland within 300m (m^2), area of arable land within 300m (m^2), area of pasture within 300m (m^2)	sett, year, individual ID

3. Results

Bait Uptake

In the analysis, data from 2010, 2011 & 2012 was used; including badgers caught from 95 setts, totalling 477 captures from 387 individuals (201 females/185 males/1 Unknown; 140 cub/337 adult captures).

Explanatory variables considered in the global model are shown in Table 2. Table 3 shows the variables included in the top models. Distance to arable land was the only variable not to be included in any of the top models (those within 2 Δ AICc confidence sets).

Model averaging indicated that bait type, area of arable land, distance to broadleaf woodland and age (in that order of effect size) had significant effects (coefficient/SE > 2; confidence intervals not overlapping zero). Sex had an effect but it was small and highly variable (coefficient/SE < 2), males showed a trend of less likelihood to have ingested biomarker (taken bait). Values for variables showing no effect are not shown.

Larger areas of arable land within a 300m radius of a sett associated with decreasing uptake (Figure 1). Increasing distance to broadleaf woodlands was associated with increased likelihood of biomarker uptake (Figure 2). Cubs were also associated with higher biomarker uptake. Finally, the bait type/packaging used significantly affected bait uptake, however discussion of this is outside the scope of the project.

Table 3. details of the subset of models with ΔAICc<2, explaining variation in the uptake of oral baits. '+' indicates the inclusion of a given parameter for each model. degrees of freedom, ΔAICc, model weight and marginal R^2 values are also included for each model.

model	age	bait type	sex	distance to access	distance to pasture	distance to habitation	distance to woodland	area of woodland	area of arable land	area of pasture	d.f.	logLik	AICc	deltaAIC	weight	R^2 (marginal)
1	+	+	+				+				8	-190.97	398.26	0.00	0.27	0.44
2	+	+	+				+	+	+		9	-190.25	398.89	0.63	0.20	0.48
3	+	+	+		+		+		+		9	-190.34	399.06	0.80	0.18	0.49
4	+	+	+				+		+	+	9	-190.60	399.59	1.33	0.14	0.47
5	+	+	+	+			+		+		9	-190.90	400.19	1.94	0.10	0.47
6	+	+	+			+	+		+		9	-190.92	400.23	1.97	0.10	0.47

explanatory variable	Estimate (Std. error)	relative importance	CI (2.5%/97.5%)
age	**0.987 (0.349)**	**1.00**	**0.303/1.67**
sex	-0.520 (0.315)	1.00	-1.14/0.0980
bait type	**3.98 (0.451)**	**1.00**	**3.10/4.87**
distance to broadleaf woodland	**1.20 (0.519)**	**1.00**	**0.192/2.22**
area of arable land within 300m (m^2)	**-1.21 (0.475)**	**1.00**	**-2.14/-0.279**

***the average model coefficients for variables in the top subset of models. average coefficient estimates, 95% confidence intervals and relative importance are displayed for each variable. variables in bold possess 95% confidence intervals which do not span zero. all predictor variables were standardised to mean zero and standard deviation of 2 prior to analysis**

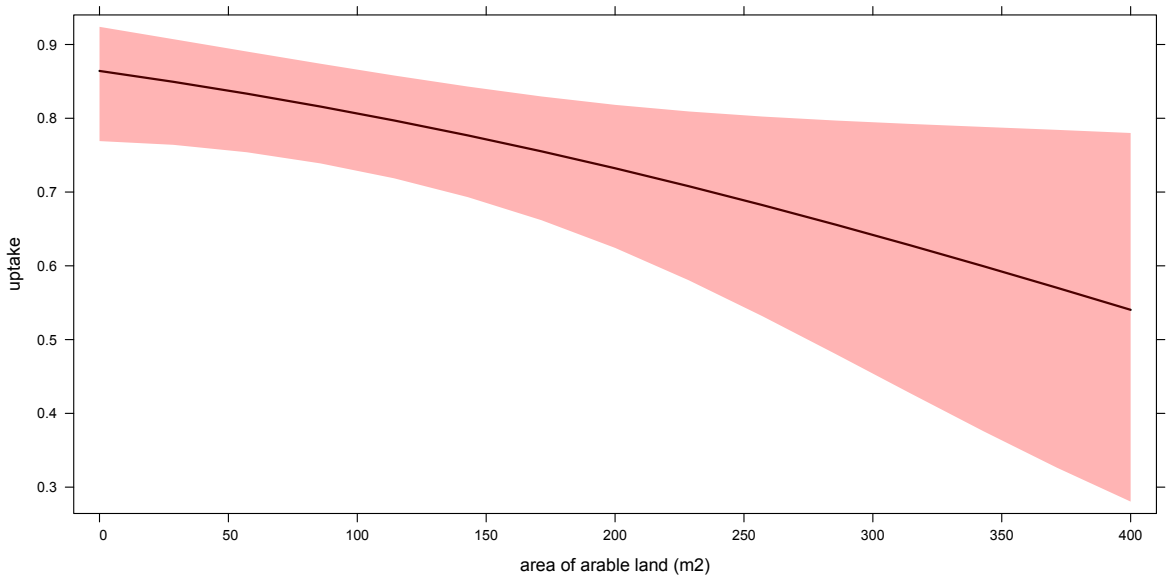

Figure 1. Relationship between the area of arable within 300m of sett and the proportion of individuals that have consumed bait. Both axes are continuous variables. The trend is based on predicted values from the second top model explaining variation, with the shaded area representing the 95% confidence interval (**Table 3**).

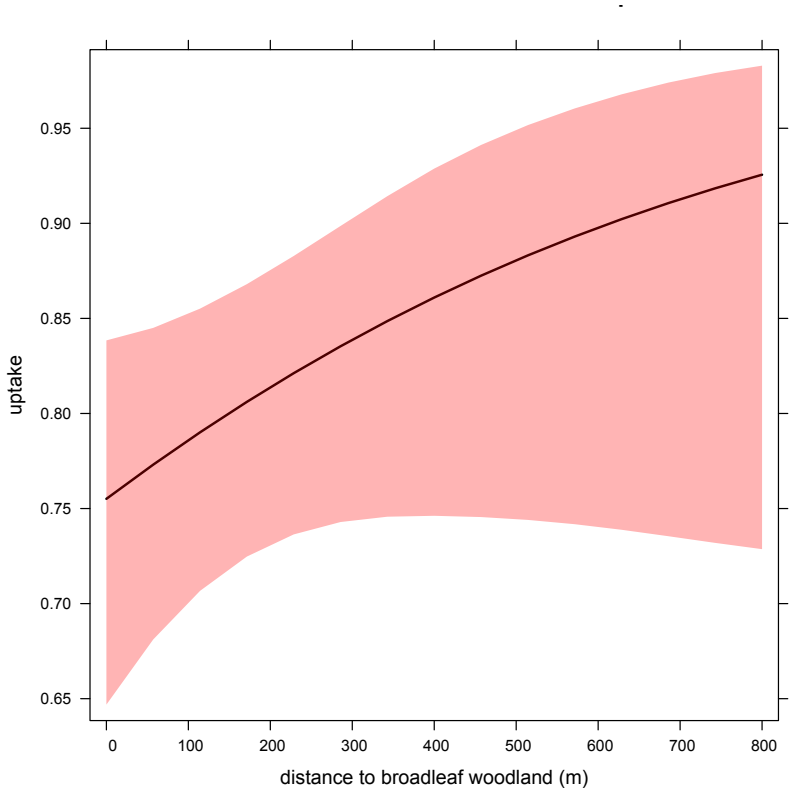

Figure 2. Relationship between the distance from the sett to broadleaf woodland and the proportion of individuals that have consumed bait. Both axes are continuous variables. The trend is based on predicted values from the top model explaining variation, with the shaded area representing the 95% confidence interval (**Table 3**).

Bait disappearance

In this analysis, data from 2010, 2011 & 2013 were used; acquired from 56 setts. Explanatory variables considered in the global model are shown in Table 2. Table 3 shows the variables included the top models. Distance to habitation was the only variable not to be included in the top models (those within 2 Δ AICc confidence sets).

Model averaging indicated that distance to broadleaf woodland from setts, the number of baits placed and the study day had significant effects (coefficient/SE > 2) (See Table 4). Values for variables showing no effect are not shown.

As shown in Table 4, with each on-going study day (Figure 3), and with higher numbers of bait deployed, there is an associated increase in the number of baits taken. Furthermore, setts further from woodland are associated with increasing levels of bait disappearance (Figure 4). Finally, the bait type/packaging used significantly affected bait disappearance, however discussion of this is outside the scope of the project.

Table 4. details of the subset of models with ΔAICc<2, explaining variation in the disappearance of bait. '+' indicates the inclusion of a given parameter for each model. degrees of freedom, ΔAICc, model weight and marginal R^2 values are also included for each model

model	day of study	bait type	distance to access	distance to arable	distance to pasture	distance to	total bait placed	area of woodland	area of arable land	area of pasture	d.f.	logLik	AICc	deltaAIC	weight	R^2(marginal)
1	+	+			+	+	+				8	-1209.58	2435.39	0.00	0.11	0.47
2	+	+	+		+	+	+				9	-1208.65	2435.59	0.20	0.10	0.49
3	+	+				+	+		+		8	-1209.97	2436.18	0.79	0.08	0.45
4	+	+				+	+				7	-1211.04	2436.26	0.87	0.07	0.40
5	+	+		+		+	+		+		9	-1209.09	2436.46	1.08	0.07	0.46
6	+	+			+	+	+		+		9	-1209.15	2436.60	1.21	0.06	0.48
7	+	+	+			+	+				8	-1210.26	2436.76	1.37	0.06	0.42
8	+	+			+	+	+	+			9	-1209.26	2436.82	1.43	0.06	0.48
9	+	+			+	+	+		+	+	10	-1208.24	2436.83	1.44	0.05	0.47
10	+	+		+	+	+	+				9	-1209.28	2436.85	1.46	0.05	0.48
11	+	+	+	+	+	+	+				10	-1208.26	2436.88	1.49	0.05	0.50
12	+	+	+			+	+		+		9	-1209.36	2437.01	1.62	0.05	0.46
13	+	+	+		+	+	+		+		10	-1208.37	2437.09	1.71	0.05	0.50
14	+	+		+	+	+	+		+		10	-1208.39	2437.14	1.75	0.05	0.50
15	+	+			+	+	+			+	9	-1209.44	2437.17	1.79	0.05	0.47
16	+	+	+	+		+	+		+		10	-1208.45	2437.25	1.87	0.04	0.51

explanatory variable	Estimate (Std. error)	relative importance	CI (2.5%/97.5%)
day of study	**0.0327 (0.00231)**	**1.00**	**0.0282/0.0373**
bait put down per day	**0.0718 (0.00576)**	**1.00**	**0.0605/0.0831**
bait type	**0.273 (0.0711)**	**1.00**	**0.133/0.412**
distance to broadleaf woodland	**0.104 (0.0453)**	**1.00**	**0.0150/0.193**

*the average model coefficients for variables in the top subset of models. average coefficient estimates, 95% confidence intervals and relative importance are displayed for each variable. variables in bold possess 95% confidence intervals which do not span zero. all predictor variables were standardised to mean zero and standard deviation of 2 prior to analysis.

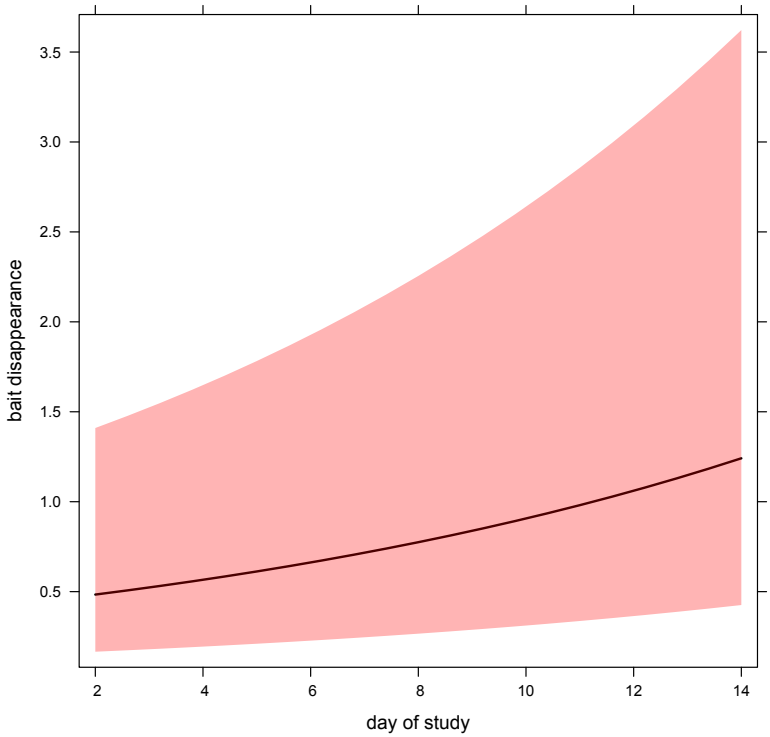

Figure 3. Relationship between the day of the study and the number of baits taken per day, per sett. Both axes are continuous variables. The trend is based on predicted values from the top model explaining variation, with the shaded area representing the 95% confidence interval (**Table 4**).

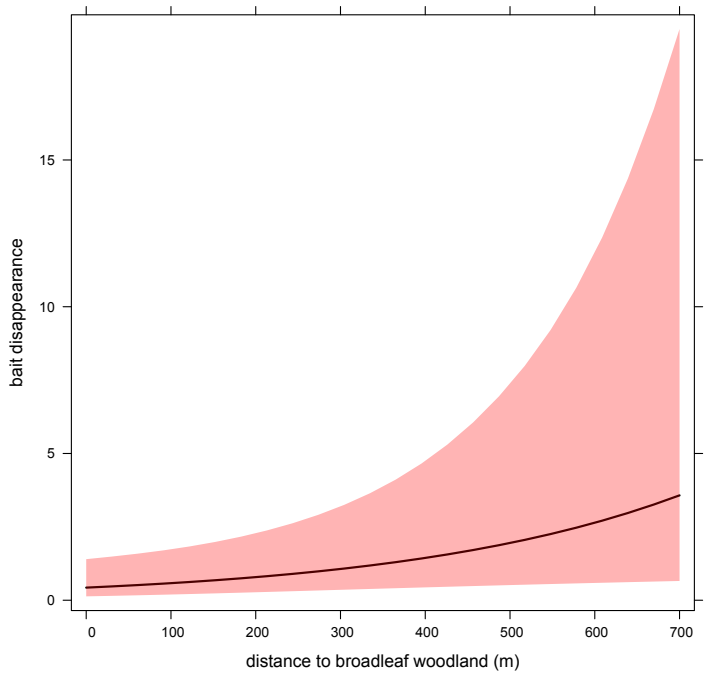

Figure 4. Relationship between the distance from the sett to broadleaf woodland in relation to the number of baits taken per day, per sett. Both axes are continuous variables. The trend is based on predicted values from the top model explaining variation, with the shaded area representing the 95% confidence interval (**Table 4**).

Table 5. details of the model with ΔAICc<2, explaining variation in the neophobia (time taken to take first bait). '+' indicates the inclusion of a given parameter the model. degrees of freedom, ΔAICc, model weight and marginal R^2 values are also included for each model

Model	day of study	distance to habitation	distance to woodland	area of woodland	distance to pasture	d.f.	logLik	AICc	deltaAIC	weight	R^2 (marginal)
1	+	+	+	+	+	8	-183.02	382.04	0.00	0.6	0.80

explanatory variable	Estimate (Std. error)	relative importance	CI (2.5%/97.5%)
day of study	**-0.831**	**1.00**	**-1.10/-0.558**
distance to habitation	**0.00796**	**1.00**	**0.00283/0.0131**
distance to broadleaf woodland	**0.0920**	**1.00**	**0.0763/0.107**
distance to pasture	**-0.200**	**1.00**	**-0.233/-0.168**
area of broadleaf woodland within 300m (m^2)	**0.695**	**1.00**	**0.0763/0.107**

*the average model coefficients for variables in the top subset of models. average coefficient estimates, 95% confidence intervals and relative importance are displayed for each variable. variables in bold possess 95% confidence intervals which do not span zero. all predictor variables were standardised to mean zero and standard deviation of 2 prior to analysis.

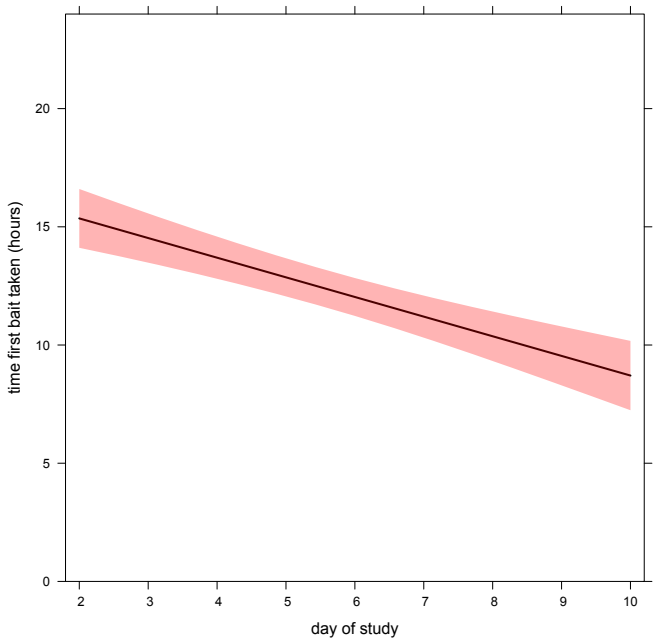

Figure 5. Relationship between the day of study in relation to the time taken for the first bait to be taken. Both axis are continuous variables, the time taken is the number of hours after midday. The trend is based on predicted values from the top model explaining variation, with the shaded area representing the 95% confidence interval (**Table 5**).

Neophobia

For the model investigating neophobia, 7 setts in Wiltshire and Gloucestershire from 2013 were used. Explanatory variables considered in the global model are shown in Table 2. There were no other models within 2 **Δ**AICc confidence sets; the values of the only top model are presented in Table 5, all of which were significant.

The strongest effect was that of study day (Figure 5), which is correlated with baits being taken earlier. Both increasing area of broadleaf woodland and increasing distance to broadleaf woodlands were associated with baits being taken later.

A trend for baits being taken later was also exhibited with increasing distances to habitation (Figure 6). Finally, the time for the first bait to be taken decreases as the distance to pasture increases (Figure 7).

4. Discussion

On-going research into the development of an oral bTB vaccine and bait deployment method suggests that the proportion of badgers in a social group that consume baits may vary between groups. The aim of this study was to provide further understanding of this variation. Table 3 shows the variables that have an effect on bait uptake.

Increasing distances to broadleaf woodland correlated with a higher uptake of baits (Figure 1). Kruuk *et al*. [45] found that badgers preferred to forage in pasture during wet weather, but used plantation and deciduous woodland in drier conditions. As the fieldwork in these studies was carried out over summer months, which are usually drier, badgers in closer proximity to pastures may have been more inclined to seek out new food sources.

Bait uptake was negatively correlated with the proportion of arable land within a 300m radius of the sett. The lower uptake may stem from a temporal abundance of arable crops, leaving bait less desirable to certain individuals. Delahay *et al*. [32] discuss the positive correlation between badger group size and the availability of arable land within the social group's territory, owed to the importance of wheat, barley, oats and maize as food sources for badgers particularly in late summer [49] This is consistent with a study by Boyer *et a*l. [47] which concluded that when targeting raccoons (*Procyon lotor*) and striped skunks (*Mephitis mephitis*), vaccine baits should be distributed later in the Autumn instead of during August when crop food availability is lower. This shows there is consistent evidence of the importance

in considering temporal effects on the uptake of oral vaccine, as previously observed in vaccination campaigns against rabies in European fox populations [24].

Age was also significantly associated with bait uptake, with cubs showing higher likelihood to have taken bait than adults. A study Ballesteros *et al*. [48] investigates ways to maximise the uptake of bait by wild boar piglets, as in a long-term deployment scheme this could be advantageous. Preferential uptake of the vaccine by younger individuals would minimise the time in which they are susceptible to infection. This could significantly improve the likelihood of the success of vaccination programmes [16].

Broadleaf woodland was the only noteworthy explanatory variable to influence the disappearance of bait. The trend observed was consistent with its influence on bait uptake i.e. as the distance to it increases; there is an increase in the level of disappearance. This again could be a down to badger preference toward broadleaf woodlands during drier periods. Neal [46] discusses how broadleaf woodlands can provide large varieties of foods in addition to invertebrates (e.g. carrion, blackberries and acorns). The availability of a more varied and familiar diet source may reduce badger's desire toward baits.

Neophobia was found to be influenced by a number of interesting local area characteristics. For instance, badgers were more neophobic (taking more to time to take baits) when a larger proportion of the sett's nearby territory was broadleaf woodland, in a similar vein to the lower uptake and disappearance of baits observed at setts closer to broadleaf woodlands.

However, somewhat conversely, neophobia was lower for setts in closer proximity to broadleaf woodland. Setts in, or nearer to, woodlands may be subject to early loss of

daylight due to tree-cover or less disturbance by human/livestock activity, which is potentially conducive to earlier emergence times; leading to quicker bait uptake. Local levels of light intensity, such as those determined by canopy cover, have been found to cause variation in emergence time [50,51]. Although there is no empirical evidence for this in badgers, studies have found this in other nocturnal species e.g. bats in roosts close to woodland exhibit earlier emergence times than those roosting in more exposed conditions [51,52]. Unfortunately, with only a limited number of cameras available it was impossible to cover badger setts sufficiently to observe and factor in emergence times.

An interesting correlation between neophobia and human habitation was discovered, with setts closer to habitation showing less neophobic behaviour (Figure 6). Badgers have been shown to take advantage of human food sources, with sett size and adult body weights showing positive correlations to nearby human food sources [31,32]. As well as a familiarisation to human food sources reducing the neophobia, the setts might be subject to more frequent disturbance, reducing unease towards baits and the bait deployment methods. This may be beneficial to bait deployment programmes, as individuals that are more likely to visit habitation (including farms, cattle housing etc.), may also be more inclined to consume novel baits than other badgers.

Bait uptake and disappearance showed no association with proximity to human habitation or access. A potential explanation is the niche diet variation among badgers [53], whereby individuals in the same social group will choose to forage in preferred areas and for preferred dietary items. Certain badgers may show a preference towards diets scavenged from human habitations, and these might be the individuals that are first/early to take bait.

The initial neophobia (as measured in the study) exhibited towards the novel baits dissipated over time. This is comparable to how captive wild rats show reduced food intake after novel bait/bait containers were introduced, but over the following 5 days began to return to normal feeding patterns [34]. The same study found that a stronger neophobic reaction is shown towards new food containers than to the novel food itself. In terms of how this affects deployment of novel bait to badgers; it shows a potential requirement for extended periods of bait placement to overcome neophobia and to maximise uptake by individuals. Pre-baiting is a method of dispensing a potentially cheaper novel food source, allowing the resident animals to become familiar with new food sources [36]. Longer pre-baiting periods were found, when baiting wild boar, to increase baiting success and the efficacy of wild boar baiting strategy [54]. Possible deployment methods could place baits directly down holes, not under slabs, to reduce aversion due to the presence of slabs.

Here we show that the local area land characteristics, of a social group's territory, does explain some of the observed variation in the uptake and disappearance of baits. Furthermore, the characteristics of the surrounding area also appear to account for some of the variation in the neophobic behaviour that badgers show towards baits/bait deployment. There is potential for further research to establish whether there are interactions between land classes and the time of year bait is deployed, this may optimise the deployment for optimal efficacy.

Peanuts and syrup have been shown to produce near 100% uptake rates [55], this was in a well-studied population where peanuts and syrup are fed annually and a bait saturation approach was taken. However, this is not a viable candidate bait, because there is a requirement for it to deliver and sustain a live, viable vaccine until it is. As the bait here has been developed as a vehicle for vaccine administration,

perhaps in populations that are naïve to novel baits, 100% uptake cannot quite be achieved.

The findings discussed here could be used to inform bait deployment, if required baits could be focused in areas where uptake is likely to be higher i.e. setts nearer habitation. Furthermore, in relation to the observed dissipation of neophobic behaviour, pre-baiting could be beneficial to maximise the success of vaccine deployment. In a wider context this paper presents interesting findings about the influence of the characteristics of the local area, both human and natural, have on animal behaviour and diet. This is potentially valuable information for consideration during the development of baiting methodologies for animals in general.

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Chapter 4. General Discussion

Chapter 4

General Discussion

Chapter 2 investigated the potential consequences of an intra-muscular Bacillus Calmette-Guérin (BCG) vaccination programme on the studied life-history traits, in free-living European badger populations. Furthermore, analyses were performed to investigate whether gamma-interferon (IFN γ) production is a potential indicator, or underpinning reason, for any changes in the life-history traits studied.

The analyses employed revealed no significant associations in vaccinated populations or individuals to any of the life-history traits investigated. Despite this, an interesting trend in IFN γ production was found; vaccinated individuals were associated with significantly lower production of IFN γ , post infection.

This was an opportunistic study, utilising data collected from a four-year parallel field-study. As such, a limitation of this study was its reliance on data collected for another purpose, the time period between data collections lowered the resolution of measurements; potentially weakening trends. Despite this the scale and overall time frame of collections still provided a dataset that had the potential to reveal more than just what was intended from the parallel study. For instance, significantly lower IFN γ production in vaccinated individuals than that seen in control individuals, is consistent with findings that post-infection magnitude of IFN γ is positive correlated with bacterial load and disease-associated pathology in a number of mammals,

including badgers [1].

Overall there was no detected trend in the influence of vaccination on the life-history traits studied. However, individuals with signs of early disease were associated with significantly higher body weights than uninfected adults. This result is difficult to explain, a past study by Tomlinson *et al.* [2] found a negative correlation between body condition and infection in adult badgers. Both studies were consistent in finding that badgers with a more progressed level of infection were associated with lower body weight/condition.

In humans, tuberculosis remains one of the major causes of infectious morbidity and mortality globally [3]. The trend seen in IFN γ production is consistent with previous studies, continuing confirmation that a interferon-gamma release assays (IGRAs) have the potential to provide more than just binary prognosis of infected/not infected. IFN γ production has already shown the potential to identify individuals at higher risk of developing active disease [1]. Further research could focus on assessing and optimising the identification of asymptomatic individuals based on their magnitude of IFN γ response.

Chapter 3 investigated whether the composition of land types within badger territories would potentially influence an oral vaccine programme. Investigating two specific areas, firstly any associations with the uptake (proportion of individuals ingesting baits) and the disappearance (total baits taken at a sett, per day) of the bait. The second aim was to investigate neophobic behaviour (tendency of an animal to avoid or retreat from an unfamiliar object or situation), whether it is exhibited and whether the territory characteristics were influential.

The analyses revealed the proximity and area of broadleaf woodland, arable land and pasture in a territory were important factors, associating with all of the tested response variables. Notably a trend in neophobia was observed, with an apparent reduction over the course of the study. Lower neophobia levels were also found to be associated to setts closer to human habitation.

The limited resources of the project allowed only a limited video coverage of each sett area, this was a limiting factor in the investigation into neophobic behaviour. Better coverage of the setts would have allowed badger emergence times to be recorded and incorporated into the analyses. This would have helped answer some of the observed patterns. The observed steady decline in neophobia is promising, as it is consistent with results from a study in rats; which showed a steady return to normal behaviour after the addition of a bait container [4].

Inglis *et al*. [4] conclude how neophobia is more pronounced with the introduction of novel bait containers than to novel bait types. Future direction for studies into oral vaccine deployment methodologies could focus on reducing the aversive behaviour evoked. If successful it may increase the number of badgers taking bait and may reduce the number of days of deployment to. This is important, as the effectiveness of an oral vaccination programme is dependent on the proportion of susceptible individuals that receive the vaccine [5], reducing the deployment time could also lower the cost involved.

For wildlife disease management to be effective, due consideration of the environment and ecology of host animal is required. The culling of badgers in an attempt to reduce disease prevalence is an example of this, as it detrimentally increases the spread of bTB in badgers, through perturbation of social groups [6].

Chapter 2 highlights how data from a vaccine efficacy trial can be further utilised to investigate the effect of a vaccination programme, in this case investigating potential changes to life-history traits of free-living badger populations. The results showed no significant associations, a promising result for a disease control method, as it was the ancillary effects of culling that were a detriment.

To be effective, the baits utilised and deployment strategies used in oral vaccination programmes must to be tailored to the target species, its diet and its ecology. The results discussed in chapter 3 emphasise this, with the local habitat as well as an instinctive aversion to novel food sources negatively impacting on consumption of bait by badgers.

Wildlife diseases can threaten biodiversity, infect humans and domestic animals, and cause significant economic losses; providing incentives to manage wildlife diseases [7]. The vaccination of badgers against bTB aims to control and ultimately eradicate bTB prevalence, among humans and livestock in the UK. BCG vaccination, both intra-muscular and delivered orally, has the potential reduce the bTB disease load in badger populations. However, it is still unknown whether this will translate to any discernable reduction to the economic or health infliction that bTB has toward humans and cattle. If no significant benefit is transferred, it cannot be a viable disease management strategy.

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Appendix: Lantra Certificate of Training in Cage Trapping and Vaccination of Badgers

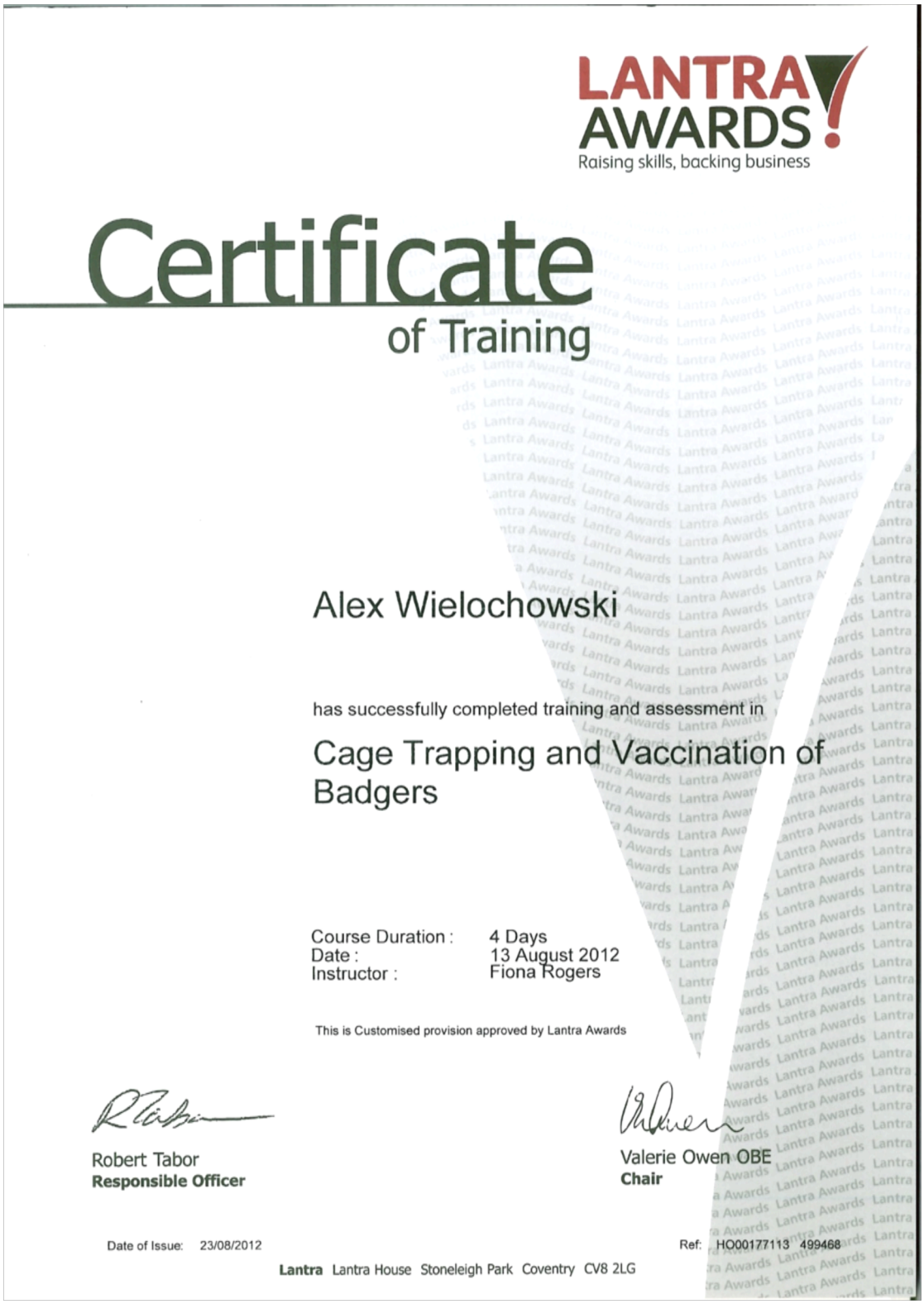
Course overview

The course aims to provide comprehensive training on the theory and practice of cage trapping and vaccinating badgers. Successful completion of the course requires passing both a written and practical assessment. Please refer to the training course leaflet for guidance on the required personal protective equipment, and health and safety. Placing traps at appropriate locations is crucial for the effective deployment of vaccine. Therefore, anyone wishing to enrol on the course must be able to correctly identify badger setts and other suitable locations, or have access to someone who can fulfil this role.

Course content

- Introduction to badgers & TB
- Licences and legal requirements
- Badger ecology
- Fieldwork theory
- Fieldwork health and safety
- Surveying for badger activity, sett checking, placement and setting of traps
- Vaccination theory
- Practical in handling vaccine and syringes
- Pre-baiting and setting traps in real trapping scenario

Course certificate



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Alex Wielochowski

has successfully completed training and assessment in

Cage Trapping and Vaccination of Badgers

Course Duration : 4 Days
Date : 13 August 2012
Instructor : Fiona Rogers

This is Customised provision approved by Lantra Awards

Robert Tabor
Responsible Officer

Valerie Owen OBE
Chair

Date of Issue: 23/08/2012

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