Developing an oral bait for badger vaccination: factors influencing bait disappearance and behavioural responses

Submitted by

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I certify that all material in this dissertation which is not my own work has 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) …………………………………………………………………………………

Table of Contents

Section one: Certificate

LANTRA Certificate of Training – Cage Trapping and Vaccination of Badgers.

Certificate
of Training

Lucy Borde

has successfully completed training and assessment in

Cage Trapping and Vaccination of Badgers

Course Duration: 5 Days
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This is Customised provision approved by Lantra Awards

Wayne Gill
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Section two: Literature Review

Managing Wildlife Diseases: A Review.

CONTENTS

I. INTRODUCTION

There is an increasing awareness that diseases in wild species can have a substantial influence on human and domestic animal health. In addition, direct effects of disease on these wild hosts are emerging as a crucial consideration in the conservation of threatened species and the management of wildlife of economic importance to humans (Wobeser, 1994; Artios *et al.,* 2001; Delahay *et al.,* 2009). In its broadest form disease in wildlife can be described as any impairment of normal functions (Wobeser, 1994; Delahay *et al.,* 2009). Non-infectious diseases include the effects of toxic substances, physiological conditions and trauma on wildlife. It is, however, the effects of infectious diseases - those transmitted between individuals, which have received the most research interest. These, often highly virulent diseases, pose the most threat to humans and consequently form the target of most disease management programs. This review will therefore focus on these infectious diseases.

Historically infectious diseases have had colossal effects on human populations, acting as the main agent of morbidity and mortality for at least the last 10,000 years (May, 1988). The 'Black Death', a plague purportedly spread by black rats (*Rattus rattus*) in the 12th century, is estimated to have reduced western Europe's population by around one third (Slack,1989) . It's shown to have created a

series of religious, social and economic upheavals greatly influencing the course of European history. Diseases such as malaria, transmitted by mosquitoes (*Anopheles spp.*), today results in over a million deaths worldwide, accounting for 20% of all childhood deaths in Africa and reducing gross domestic product by as much as 1.3% in countries with high disease rates (WHO, 2010a).

Diseases, particularly those infectious in nature, pose a substantial threat to the conservation of global diversity (Daskek *et al.,*2000). Disease is commonly listed as a serious threat facing many endangered species such as the Giant Panda (*Ailuropoda melanoleuca*) (Zhang *et al*., 2007), and the Black footed ferret (*Mustela nigripes*) (Thorne & Williams 1988) and has been suggested as the cause of extinction of the Sharp-snouted Day frog (*Taudactylus acutirostris*) (Schoegel *et al*., 2006).

To facilitate an adaptive management approach for future wildlife disease management, whereby we learn from previous experiences to develop better strategies, this review aims to synthesize knowledge on key concepts of disease dynamics and collate and evaluate the efficacy of different management strategies employed to tackle these diseases. Before undertaking any form of wildlife disease management several factors should be taken into consideration; clear objectives and aims must be decided, knowledge of the most appropriate target for management and which practical method is most suitable and effective. Understanding the epidemiology of diseases will be crucial in achieving these goals.

II. WHY MANAGE DISEASES IN WILDIFE?

There are three main drivers behind the justification of management of such diseases: Threats to; 1) human health, 2) domestic livestock or wild species of value to humans and 3) species of conservation concern.

(1) Threat to humans health

Most major human infectious diseases have animal origins (Wolfe *et al.,* 2007). Certain infections are caused by direct animal-to-human transmission, such as rabies. Others have become independently sustained within the human population, so that the causative virus has diverged from its animal progenitor, as in the case of measles (Weiss, 2001). The magnitude of such diseases can range from a handful of cases of human illness, to vast epidemics infecting millions of people across many continents. There have been several epidemics of sleeping sickness, transmitted by the tsetse fly (*Glossina* spp.)

over the last century. In 1998, 40,000 cases of this debilitating disease were reported in Africa, however it was estimated that between 300,000 and 500,000 more cases remained undiagnosed (WHO, 2010b). The recent, widely documented epidemics of Severe Acute Respiratory Syndrome (SARS) and highly pathogenic avian influenza (HPAI) H5N1, have acted to highlight the severe health and socioeconomic impacts that emerging infectious diseases can have on human society, and have also re-emphasized the importance and interest of zoonotic origins for many diseases (Desselberger, 2000; Weiss, 2001). Additionally a previous proposal by the United States to ban blood donation by persons who have spent longer than 6 months in the UK during 1980-1996 and therefore are considered carriers of the BSE agent, would reduce U.S blood supply by 2.2% (Daskek *et al.,* 2000), further demonstrating the vast temporal and spatial indirect consequences that diseases can have on humans.

(2) Threat to domestic or wild animals of value to humans

Diseases of domestic species, especially in intensive farming systems, can be severely debilitating, reducing productivity and resulting in serious economic losses for individual farmers (Thomson *et al.,* 2003). Countries or regions, in which particular diseases do not occur, restrict imports of livestock or their products from countries where the disease is prevalent. In 2004 the United Nation's Food and Agricultural Organization reported more than one third of global meat exports were affected by disease outbreaks such as Bovine spongiform encephalopathy (BSE) and Avian Influenza (FAO, 2004). In 2009, 25,557 cattle were compulsory slaughtered for bovine tuberculosis (bTB) control in England, with governmental spending of approximately £63 million in 2009/10 to tackle this disease (DEFRA, 2011).

It is often extremely difficult to quantify the contribution of transmission of disease from wildlife to domestic species and vice versa. However the existence of 'Wildlife Reservoirs', where a disease is maintained by that wild species or population, are often implicated as the main limiting factor affecting control of certain diseases in domestic species. Kaden *et al.,* (2000) reported that 52% of all outbreaks of Classical swine fever (CSF) among domestic pigs were caused by direct or indirect contacts with infected wild boar (*Sus scrofa)*. Wild plants can also serve as sources or reservoirs of viruses that induce crop diseases; Hepperly, Kirkpatric & Sinclair (1980) isolated three fungal pathogens of soybean (*Gycine max*), a commercially important crop, from a common weed found in soybean fields.

An additional impetus for managing disease occurs when wild species are of economic value to humans. For example viral diseases shared between domestic and wild flocks of birds can affect economically relevant game birds e.g. avian pox in red-legged partridge (*Alectoris rufa*)(Millán,

2009). Furthermore, for those countries that increasingly rely on wildlife for generating ecotourism, disease affecting wild animals may have detrimental effects on income generation.

(3) Threat to species of conservation concern

Infectious and non-infectious diseases are being increasingly recognized by conservation biologists as a challenge to the preservation of wildlife. In the case of squirrel Poxivirus, pathogen-mediated competition between an invasive species, the grey squirrel (*Sciurus carolinensis)* and the native Eurasian red squirrel (S*ciurus vulgaris*), results in the rate of red squirrel replacement by grey's being some 20 times faster in those areas where grey's carry the virus (Gurnell *et al.,* 2006). Metapopulation theory suggests that small and fragmented populations of endangered wildlife are more prone to extinction through stochastical events such as disease outbreaks (Gortazr *et al*., 2007); the loss of endemic Hawaiian avifauna due to the introduction of avian poxvirus and avian malaria is well documented (Warner, 1968; Atkinson *et al*., 2000). Furthermore Zhang *et al.,* (2007) suggest that the giant panda, a global symbol of wildlife conservation, is currently most significantly threatened by disease caused by an ascarid nematode. Additionally the characteristic reduction in genetic variation of small isolated populations can hinder the ability of such populations to survive disease events and even if genetic variation for resistance to a certain disease is present, rapid reduction in population size due to disease may lead to extinction before selection can change the population's genetic composition and allow it to increase in numbers (Alexander *et al*., 1996). The persistence of a pathogen in the environment is an important prerequisite for pathogen exposure and the continued presence of a biological reservoir fulfils such a criteria (Alexander *et al*., 2008). Reservoirs of disease in domestic species can pose a significant risk to wildlife species that can contract the disease. The spread of infection from one host species, where it appears to be endemic, to another in which it cannot persist, highlights the importance of reservoirs. For example in the Serengeti the domestic dog acts as a reservoir of rabies infection in Side striped jackal (*Canis adustus*), who appear unable to sustain endemic infection without the transmission from dogs (Hudson *et al*., 2002).

Parasites are natural components of any ecosystem and influence the structure of ecological communities, for example, heavy lungworm infestations occur in roe deer (*Capreolus capreolus*) in some parts of the UK and the parasite probably acts as a form of biological control in this species (Simpson, 2002). Additionally, pathogens are implicated as important drivers of evolutionary change (Wobeser, 2002) and this raises the question of whether humans should intervene with diseases in wildlife. Human modification of the environment is so widespread and considerable that the ability to distinguish a natural from human influenced disease event is greatly compromised. Disease

management in wildlife could therefore be viewed as an attempt to mitigate the consequences of human actions (Wobesser, 2002). The decision of when and when not to intervene with disease management strategies essentially rests on the extent to which the disease endangers human health, wealth or conservation aspirations, and the likelihood that the intervention will have a beneficial effect.

III. INFORMATION NEEDED TO MANAGE DISEASE

Several factors influence the spread and persistence of a contagious agent once it has been introduced into a host population; the infectiousness and pathogenicity of the disease, the average time the infected host is infectious, the density of susceptible hosts and the frequency of host contact (Deem *et al.,* 2001). Additionally, the ability of humans to detect and monitor diseases will affect their magnitude and subsequent effects.

(1) Agent dynamics

The division of diseases into 'non-infectious' and 'infectious' diseases can be further partitioned based on the differing dynamics of infectious diseases, based on whether 'micro' or 'macro' parasites are responsible for its development. This partitioning highlights common dynamics of disease, such as likely sources, or necessary thresholds for infection or persistence, which need to be understood to effectively tackle the disease. For example, in sexually reproducing macroparasties there is a persistence threshold below which parasite density is too low for mates to find each other within the host; the resulting mating failure hinders the spread of the parasite in the host population (Deredec & Courchamp, 2003). The rate at which a parasite multiples and increases within a host population is important for disease management. A certain reproductive rate (R_0) must be achieved for a parasite to be maintained in a population and the greater the R_0 the more likely the disease will spread within a population (Wobesser, 2002). R_0 can greatly influence the epidemiology of an infection, affecting invasion success of the pathogen, its persistence and patterns of disease dynamics (Hudson *et al.*, 2002). Disease can affect individual hosts in a variety of ways: reducing growth rates or fecundity, increasing metabolic requirements, changing patterns of behavior, or resulting in mortality. The course of the disease within the individual host will determine the dynamics of excretion of the infectious agent and the rate of disease induced mortality, and by combining these factors the output and duration of the infectious period can be identified (Delahay *et al.*, 2001).

10

(2) Host dynamics

The demography of host populations will also greatly influence the dynamics of disease. Host population density thresholds for the invasion or persistence of infectious diseases are central concepts of disease ecology and underline disease management polices based on depopulation or vaccination (Lloyd-Smith *et al.,* 2005). Factors such as reproductive strategy, territorial behavior or seasonal migration will affect disease transmission; it is widely recognized that seasonal breeding affects the pattern of input of susceptible individuals into a population, and species that mark territories using scent marking, are likely to have increased contact rates with parasite-laden faeces (Cross *et al.,* 2007*)*. Hosseni *et al. (*2004) developed a theoretical model of disease dynamics, investigating how several seasonal factors: births, aggregations and variation in immunity affect dynamics of House finch (*Carpodacus mexicanus*) conjunctivitis in North America. They document how both latitudinal variation in the timing of breeding and social systems could explain the dynamics of the house finch-conjunctivitis system. In particular, while either alone is sufficient to create the recurrent cycles of prevalence in a population with an endemic disease, both are required to produce the semi-annual pattern of disease prevalence seen in this disease system. This further suggests that variation in social structure could, and probably does, interact with variations in immune function and the endocrine systems.

Spatial structuring of the host population may also greatly influence the pathogen persistence. If the population is divided into sub-populations a pathogen may invade a host then 'jump' onto another susceptible population, leaving the original populations to recover through immigration and birth of susceptible animals, and once again become suitable for future invasion. This spatial dynamic has been suggested to provide the key to understanding the non-persistence of phocine distemper virus (PDV) in harbour seals (*Phoca vitulina*), where within the population the epidemic ceases before sufficient new susceptible were introduced at the annual pupping season (Hudson *et al.*, 2002). It must also be noted that there may be heterogeneities in host susceptibility which could also significantly affect transmission rates: it has been suggested for human diseases that age structuring is important, whereby disease transmission is more likely between similar ages than between age groups (Anderson and May, 1985). In another example a longitudinal study of the Cowpox virus in wild wood mice (*Apodemus* sylvaticus) and bank voles (*Myodes glareolus*) (Hazel *et al.,* 2000) documented that infection was most common in males of both species, but could demonstrate no association with age. Differences in aspects such as immunity or behavior in response to pathogen exposure may be evident not only between but also within species (i.e. Zuk & Stoehr, 2002), complicating the prediction on the efficacy of different management strategies.

(3) Surveillance and monitoring

An important prerequisite to achieving effective management of any disease is surveillance. The ability to detect a disease and monitor its progress within individuals, populations and communities will aid practical reactive management. Furthermore predictions of disease dynamics based on observed patterns and correlates will assist proactive strategies, potentially reducing the impact of the disease. It is accepted that countries that conduct disease surveillance in their wild animal populations are more likely to detect the presence of infectious diseases, and thus act more swiftly to adopt countermeasures (Morner *et al.*, 2002). In contrast to our knowledge of humans and domestic species, baseline data on the 'normal' disease prevalence for many wild species do not exist (Deem, *et al.,* 2001). Capture, sampling and marking of individuals has been employed to monitor changes in disease prevalence. In the case of Devil facial tumour disease (DFTD) in Tasmanian devils (*Sarcophilus harrisii*), permanent marking using ear tattooing or more recently microchip insertion allows information on population changes in the presence of DFTD prevalence, and mortality rates to be gathered (Hawkins *et al.*, 2006). In the case of emerging diseases, geographical spread could be assessed by monitoring sites at varying distances outside the disease front. Additionally regular monitoring would be advisable to detect the relative timing of any changes in prevalence. Childs *et al*. (2007) investigated the suitability of passively collected surveillance data to determine the presence or absence of rabies in the United States. The size of human population and total expenditures on testing within counties accounted for 72% and 67%, respectively, of the variance in testing. This led to the recommendations that active surveillance may be required in locales with sparse human populations when a high degree of confidence in the status of rabies is required. Dispersal behaviors of hosts are critical in determining patterns of disease spread, yet are often challenging to measure in wild populations. Novel approaches are developing alongside more traditional techniques (i.e. radio telemetry, capture-mark-recapture) for example estimates of raccoon (*Procyon lotor)* dispersal were obtained through parentage and spatial genetic analysis (Cullingham *et al*., 2008).

IV. TARGETS AND METHODS OF MANAGING DISEASE IN WILDLIFE

Disease management can be of three basic types: Prevention, Control or Eradication. Prevention involves precluding the occurrence of disease from individual animals, populations or areas where it does not already occur. Control acts to reduce the frequency of occurrence or the severity of existing diseases and Eradication aims for the total extirpation of a disease from a population or area (Wobeser, 1994). Which of these management strategies is employed depends on several factors

including the availability of techniques to detect, diagnose and manage the disease (Wobesser, 2002).

Different types of diseases demand different strategies. In the case of non-infectious diseases the target of management will be to limit access to the risk factor and eliminate this source of risk. For infectious diseases management may be attempted by manipulating any of the three basic determinants of the disease: the agent, the host, or the environment, and also by influencing human activities.

(1) Targeting the Agent

The aim of targeting the agent is to directly reduce the reproductive rate of the pathogen. Vaccinating against a disease aims to limit the number of susceptible individuals in a population. Alternatively treating an already infected individual aims to reduce the intensity or duration of the infectious period, therefore reducing the number of infectious animals present in a population at any given time. The use of medication to treat diseases is largely restricted to rehabilitation or translocation exercises. Western barred bandicoots (*Perameles bougainville*) were screened for eye infections prior to a captive breeding program and treated if necessary (Gortazr *et al.,* 2007). This technique of health screening is now well practiced before wildlife re-introductions (i.e. Mathews *et al.,* 2006); however it must be noted that, in many cases, the actual effectiveness of these treatments is unclear. Treatment is less common in wild populations of animals due to the fundamental practical difficulties of delivering medication to wild animals (McCallum, 2005) and the cost of doing so. Treatment of disease is increasing in use for valuable animals, such as game species or engendered animals of conservation concern. The treatment of bighorn sheep (*Ovis canadensis canadensis*) with chemotherapeutic drugs was trialed in a small population in the 1970s (Schmint *et al*., 1979). By administering pregnant ewes with Fenbendazole, a reduction of lungworm infestation and a subsequent increase in lamb survival was documented. However a more recent study, trialling the same drug, reported no demonstrable improvements in lamb survival (Miller *et al*., 2000). The lack of appropriate drugs for most wildlife species and different individual level responses to these drugs may limit the suitability of treatment of a strategy to tackle disease in wildlife.

Vaccinating animals aims to disrupt the transmission of a particular disease. Vaccines contain antigens associated with the target pathogen, which upon entering the individuals body, invokes and immune response, without causing disease. Different methods, such as trapping, darting or netting are used to capture and directly vaccinate animals. Serum samples collected prior to and following vaccination revealed that 100% of striped skunks (*Mephitis mephitis*) and 98% of raccoons

vaccinated against rabies successfully seroconverted (Rosatte *et al.*, 1990). However questions over direct vaccination have been raised. It has been suggested that handling of animals may adversely affect the survivorship of these individuals during or post-release (Kock, *et al.,* 1987), however it is the substantial resource requirement, of time and money, which limits the feasibility of this approach.

The use of oral vaccination has emerged over the last 15 years as an alternative method of vaccine delivery, mainly driven by the success of wide scale oral rabies vaccination programs for meso-carnivores in North America and Northern Europe (Cross, *et al.,* 2007). The first field trials using recombinant vaccinia virus rabies vaccines from the late 1980s- early 1990s reported the delivery of approximately 8.5 million field doses in northern Europe and coincided with a dramatic reduction in rabies incidence in red foxes (*Vulpes vulpes)*, the main target species. However, the targeted management of rabies in North America is presenting greater challenges due to the wide diversity of host species (Cross, *et al.,* 2007).The successful use of an oral wildlife vaccine is dependent on a combination of three key components: an efficacious immunogen, a suitable delivery vehicle, and a species-specific bait. For example BCG needs to be delivered as a live, replicating immunogen in order to generate effective protection and it is crucial that the immunizing bacilli remain viable during oral bait delivery. BCG has been incorporated into a lipid matrix, which when solidified, stores the bacteria in a live state for weeks in ambient temperature (Cross, *et al.,* 2007), a highly desirable trait for a vaccine to be distributed in field. Differing food preferences and foraging habits of the host target will affect the attractiveness of oral baits to different species/age classes. A study testing the effectiveness of immunization of wild boar against CSF, rate of uptake of oral baits was determined to be between 85-100%, with antibody prevalence reaching 49-50% after immunization (Kaden *et al.*, 2000). However this study also documented that >50% of young boars did not feed on vaccine baits nor become immunized. A trial combining a CSF vaccine into the meat baits concluded the high probability of the baits and hence vaccine to be eaten by other non-target species (Kaden *et al.,* 2000).

The effectiveness of a vaccination program will depend on the proportion of animals that receive the vaccine and the proportion of those individuals that become immunized (Delahay *et al.*, 2009). Some limitations and drawbacks to the use of vaccination have been raised. The age at which susceptible

animals are exposed to the disease agent is an important consideration. For vaccination to be successful immunization must occur prior to infection (Wobesser, 2002). This means that diseases to which young are exposed, or when young are hard to access (i.e. badgers (*Meles meles*) in setts), are inherently harder to control. Smith & Wilkinson (2003) modeled the control of rabies outbreaks in red fox populations. They reported that vaccination had greatest control success if implemented immediately after cubs were born, but concluded that vaccination was less successful than culling, and the lack of success was related to the birth of rabies-susceptible young in spring. However, vaccination is increasingly being viewed as an publically acceptable and functional strategy (Delahay *et al.,* 2003; Waters *et al.*, 2004) and it is widely recognized that oral delivery is the most practical means of vaccinating wildlife (Knobel *et al.,* 2002; Jojola *et al.*, 2007). Further progress in the field will be generated through development of more effective immunogens, delivery systems and species selective baiting systems (Cross *et al.*, 2007).

(2) Targeting the Host

Infectious diseases have geographic range determined by the presence of suitable hosts and various environmental factors that allow the transmission of the infectious agent (Wobeser, 2002). Epidemiological theory predicts that for directly transmitted infections, per capita rate of disease transmission and prevalence of disease will increase with increasing population therefore targeting the host normally involves some form of population density management: altering animal distributions, removal of individuals (culling), or control of reproduction.

Culling, through variety of methods such as poisoning, gassing or shooting is the subject of intense scientific and social debate. Following Lord Krebs' 1997 review of TB in cattle in badgers, the Independent Scientific Group (ISG) undertook a Randomised Badger Culling Trial (RBCT), extending over 3000km2 and a 10 year time span (ISG, 2007). 'No' culling, 'Reactive' culling, whereby all badgers whose territories includes a bTB breakdown farm were removed and, 'Proactive' culling , the widespread clearances of large defined areas, were compared. The RBCT reported that whereas proactive culling could reduce incidence within treatment areas by 23%, reactive culling led to rapid increases of 20% in TB incidence. Additionally they found that peripheral farms to the culling area experienced rates of cattle TB incidence 29% higher than controls. As culling badgers brought both positive and negative effects for bovine tuberculosis incidence in cattle, it was concluded that this management strategy could not make a meaningful contribution to the control of this disease. Only with knowledge of badger social structure, could the results from this trial be elucidated. Badgers in the UK have developed highly complex and stable social structures, with social group territories (Rogers *et al.*, 1998). However by perturbing this social structure, residual badgers that escaped

culling, or those living on the periphery of the RBCT culling area, increased the frequency and distance of ranging behaviors, potentially exacerbating disease spread to other badger and cattle (Woodroofe *et al.*, 2006; Carter *et al.*, 2007; McDonald *et al.*, 2007).

A crude belief that killing half the wildlife population will halve the risk of infection does not take into account complexities such as density-dependent process that act on the birth and death rate of the host population. However these processes will affect the efficacy of different control strategies. As in the case of CSF in wild boar populations, selectively hunting older immune individuals results in compensatory birth rates, in-turn increasing the number of young susceptible individuals in the populations and potentially promoting transmission of the disease (Husdon, 2002). The presence of multiple hosts for a pathogen adds complexity to a disease system. Multi-host pathogens can infect a wide variety of wild and domestic species, increasing the potential for pathogen maintenance in a system through interspecies transmission. For example there is strong circumstantial evidence that a herd of African buffalo (*Syncerus caffer)* introduced into a Zimbabwean conservancy infected antelope in their vicinity with foot and mouth disease (FMD) virus, and these antelope had subsequently transmitted the infection to cattle outside the conservancy fence (Hargreaves et al., 2004). In multi-host situations, the management of all hosts may be required to effectively tackle a disease system.

Selective culling of diseased animals may increase the effectiveness of lethal depopulation to decrease disease prevalence, however problems with diagnosing diseased from healthy animals hinders this approach. For example chronic wasting disease (CWD) in deer and elk (*Cervus canadensis)*, initial symptoms emerge as subtle behavioral, rather than physical changes. Inspection of game herds by regulatory animal health officials often fails to detect evidence of CWD and Williams & Miller (2002) conclude that the only reliable method for detecting CWD-affected populations is through microscopic evaluation of appropriate tissues. Gender -specific physiological and behavioral traits can differently affect disease transmission and hence prevalence (Smith, *et al.,* 2001). For example bTB prevalence in Michigan white tailed deer (*Odocoileus virginianus)* differs significantly between males (8%) and females (2%) (O'Brien *et al.,* 2002) and Fenichel & Horan (2007) suggest targeted harvesting on this observable trait correlated with infection.

If disease eradication is the aim of management then the population density must be reduced to level below which infection can be maintained. However it may be more realistic to reduce population density to a level where spill over of a disease to other host species (such as domestic animals or humans) does not occur. Artios et al (2001) argue that in the long term, if an infection pressure persists, then culling a species will not be economically sustainable. Furthermore predictions on the effect of depopulation are hard when proposing radical changes in population

density when the system has not been previously observed at a wide range of densities (Hudson *et al.,* 2002). Using culling as a sole approach to disease management in wildlife appears only to be feasible in a small subsection of disease instances for example island populations where geographical barriers limit animal dispersal, or to cope with point-source wildlife disease outbreaks (Gortazr, 2007).

Fertility control could be employed as a non-lethal approach to reducing the density of a target population. This relatively new approach to manipulating wildlife populations has gained momentum in several human: wildlife conflicts (Seal, 1991; Bromley & Gese, 2001; Fagerstone, 2002) and is now emerging as a practical consideration for managing disease interfaces. Fertility management acts to reduce the host population through decreasing birth rate and therefore reducing the input of susceptible individuals into the population. Malcolm *et al*., (2010) recently investigated the use of intrauterine devices in white-tailed deer, and demonstrated their ability to reduce pregnancy rates. Chemical contraception through the use of synthetic steroids, oestrogens and progesterones or using immunological vaccinations can act to alter a suit of reproductive processes, including mating behavior, gamete production, maturation and fertilization, or embryonic development (Andrabi & Maxwell, 2007). Bovine brucellosis is a bacterial disease transmitted between animals such as elk, bison and cattle, primarily through contact with aborted fetuses, placentas and parturient fluids. Miller *et al*., (2004) demonstrated the ability of a gonadotropin-releasing hormone vaccine to reduce pregnancy in bison and thereby providing a potential means to prevent transmission of this disease.

The feasibly of fertility control is often impeded by the lack of understanding of crucial reproductive behaviors and physiology of many species (Fagerston, 2002) and also faces the same delivery challenges as other vaccination approaches (Buddle *et al.*, 2000). Several authors have concluded that fertility control may be less effective in reducing population density than lethal control as can only act to decrease the number of one age cohort at a time (White *et al.,* 1997; Tuyttens & Macdonald, 1998) and is also less effective in reducing transmission as only removal susceptible individuals from a population, whereas culling also acts to remove infectious animals. Culling however, in certain countries results in vocal public opposition whereas acting to reduce birth rates appears to be perceived by the public as more humane and morally acceptable which may provide the impetus for further research and development and political support in this area of disease management.

(3) Targeting the environment

Managing disease through alteration of elements of the environment, other than the causative agent or host population, is based on the concept that disease is a result of the interactions among agent, hosts and environment; in this concept a disease may only occur when certain environmental factors are present (Wobser, 1994). Alexander *et al., (*2008) reported that outbreak boundaries of CDV in African wild dogs (*Lycaon pictus*) were found to coincide with ecotones (transition zones between habitat types) rather than the spatial distribution of contiguous packs. This emphasizes the importance of landscape heterogenties in disease transmission and the potential strategic use of ecotone manipulation in conservation land use planning for this endangered species. Environmental features may also be targeted for management, for example, infection of impala (*Aepyceros melampus*) with FMD has been documented along the path of a water course where animals aggregate and experience increased transmission rates (Thomson *et al.,* 2003).

The physical segregation of wildlife from domestic species has been adopted at disease interfaces. For example in southern Africa, minimizing the effect of Foot and Mouth disease on the international trade of beef and other livestock products, has largely been achieved by segregation of wildlife and livestock using fencing (Thomson *et* al., 2003). However the use of a physical barrier has been increasingly criticized by conservationists as they can act to block migration routes and access to water /food sources. Other simple manipulations have been applied in urban situations to reduce the potential transmission of diseases from co-habiting wildlife and humans. Increased abundance of several species of gulls (*Larus* spp.) has resulted in increased risk of transmission of parasites through contamination of water sources. Several architectural and habitat management approaches, such as the covering of landfill refuse and overhead wires on roof structures, are currently employed to reduce gull/human conflicts (Belant, 1997). However the authors importantly warn of the danger of uncoordinated management efforts which could cause the relocation of problems to surrounding areas.

The development of early warning systems utilizing environmental correlates of disease outbreaks has been suggested. Nan *et al.,* (2009) report that in aquaculture systems, fish disease occurrence is mainly caused by water quality problems, such as high concentrations as nitrogen and phosphorous, and suggest monitoring these elements could allow disease outbreaks to be pre-empted and prevented.

(4) Targeting Human Activities

Although disease is a natural part of any ecological system, such widespread anthropogenic habitat alterations has led to the dissolution of many ecological barriers important in the natural control of disease (Polley, 2005). Anthropogenic actions can be implicated in changes in disease ecology (Deem 2001). The loss of endemic stability is one example of such as shift in disease ecology. An endemically stable disease is one in which the agent, host and environment coexist in a manner that results in the virtual absence of clinical disease. Ecological change provides an opportunity for pathogen, host and the environment to interact in novel ways and can result in the emergence of disease (Alexander *et al.,*2008). In many regions of the world, diseases that were previously endemically stable are now unstable due to anthropogenic change (Deem *et al.*, 2001). The loss of habitat due to human activities has led different species to exploit alternative resources, for example storks and kites foraging on rubbish dumps (Gortazr *et al.,* 2007), and thus creating another interface for human or domestic species pathogens to become established in new wildlife hosts. Conversely supplementary feeding (e.g. maize feeding of white tailed deer) act to increase population densities result in aggregations of animals, increasing the risk of transmission.

Many wild populations are subject to various harvesting regimes. Although the effects of parasites on host population dynamics is well established (Anderson & May, 1981) infectious diseases are have rarely been accounted for in harvest models. Choisy & Rohani (2009) present a model in which they demonstrate that the interaction between density dependent effects and harvesting can substantially increase both disease prevalence and the absolute number of infectious individuals. Furthermore the effect of the timing of the harvest season on the epidemic amplitude of diseases was explored. Specifically, they show that hunting just before the epidemic peak dramatically decreases the epidemic amplitude, whereas hunting just after the epidemic peak can substantially increases the amplitude. Therefore it should be ensured that knowledge of the effects of the timing of harvesting is known by hunters, or introduce necessary restrictions surrounding disease outbreaks.

Simple actions such as changing domestic species management practices, has potential to reduce disease prevalence in wild and domestic animals, and transmission between them. Density of horses turned out together was positively associated with the risk of exposure to Leptospirosis (Barwick *et al*., 1998), and cattle have been observed to avoid pasture contaminated with badger urine or faeces, except at high grazing densities (Scantlebury *et al*., 2004). Furthermore systematic studies from field experiments (Garnett *et al.* 2002; Garnett *et al.* 2003; Roper *et* al. 2003; Tolhurst *et al.* 2009) using radio tracking, camera surveillance and faecal analysis have revealed the regular use of cowsheds, feed stores, barns, and cattle troughs by badgers. Therefore to reduce the transmission

risk of bTB between these two species educating farmers of the importance of farm bio-security measures could have significant effects of transmission rates of this disease.

The increasing trend in eating meat, fish and mollusucs raw or undercooked facilitates a number of macroparasite caused zoonoses (Macpherson, 2005). For example the cod worm (*Pseudoterranova decipen*) is a nematode found abundantly in marine fish such as cod and pollock and can be transmitted to humans if these fish are eaten raw as they traditionally are in Japan (Oshima, 1987). Educating humans and guiding change in consumption patterns could help reduce zoonotic diseases such as these. Understanding the effect that human actions, targeted at other species, have on the prevalence of a disease is also important. The cod worm nematode is also a ubiquitous nematode of pinipedia, and the protection of marine mammals in the 1970s, resulted in an increased population of seals and porpoises, which increased the abundance of nematodes in the environment and consequentially in edible fish (Oshima, 1987).

(5) Integrated approaches

All the above management methods have theoretical and empirical limitations, and a combination of several of these options may prove most successful, as disease systems often involve complex interactions between many components. However, few experiments in natural conditions have explored such multifaceted approaches. The poor uptake rate of oral baits by young boars resulted in authors suggesting intensive hunting of this age group as a necessary adjunct to the use of vaccine for the older age class (Kaden *et al.*, 2000). Modeling work focused on high-density populations of rabies-naïve fox populations in the UK, suggest that a combined strategy of central poison baiting, with a ring of vaccine bait could be employed to successfully eradicate an outbreak of the disease (Smith 2003). Gurnell (2006) also using modeling techniques suggest landscape scale strategies for managing Squirrel Poxvirus disease threat, employing a suite of different management techniques. They conclude that the conservation of red squirrels will depend on minimizing contact between red and grey populations i.e. maintain red squirrel refuges (such as Kielder Forset, Northumberland, UK), with grey squirrel free buffer zones. This strategy would require effective monitoring and selective removal (through trapping or shooting) of grey squirrels at invasion pinch points in the landscape. Furthermore through analysis of key habitat preferences for grey squirrels, the authors suggest that carefully designing a suitable mix of tree species would decrease the incursion of grey into red squirrel habitat, thus reducing transmission risk.

V. CONCLUSION

The efficacy of different management strategies to prevent, control or eradicate disease will vary according to differences in variables relating to agents, hosts or the environment. Lessons can be learnt from both successful and failed interventions and will aid the formation of an adaptive knowledge base, from which future strategies can be developed.

Historically management decisions have been based predominantly on the opinions of experts, following no established procedure. However this process is changing, with increasing numbers of international meetings allowing consultation and collaboration between experts from different disciplines and across many countries, there is now a growing awareness of the need to develop a clear framework for the management of wildlife diseases based on scientific evidence (Artios, et al., 2001).

However despite advances in modeling and statistical analysis of diseases in wildlife Thompkins (1998) reports a general consensus a large gap between theory and solid experimental evidence. When management becomes necessary may vary widely between stakeholders, with differing perspectives and priorities. Therefore it is also crucial that diverse groups of stakeholders are engaged in decision making to develop strategies collaboratively. The success or failure of a disease management program is determined largely by cost, practicability and efficiency and it is essential to base policies for wildlife disease management on sound science with consideration of ethical and socio-economic issues.

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Section three: Research Project

Developing an oral bait for badger vaccination:

factors influencing bait disappearance and

behavioural responses.

Abstract

The Eurasian badger (*Meles meles)* is believed to constitute an important wildlife reservoir of bovine tuberculosis (*Mycobacterium bovis*), and is wildly implemented in transmission of the disease to cattle. Recent work has demonstrated that Bacillus Calmette-Guérin (BCG) vaccination can induce a significant protective effect in badgers, with current research focusing on the best method of vaccinating wild badgers. Oral vaccination, using baits containing the BCG vaccine, is widely accepted as the most practical and desirable method of vaccinating a large number of badgers over a wide geographical area. This study acted to investigate the factors influencing the number of baits taken by badgers, and the behviour badgers exhibit towards baits, as the success of a vaccination campaign will be greatly affected by the number of badgers consuming vaccine laden baits. Using video surveillance we identified that badgers show preference behaviour, with strong smelling bait eliciting high levels of investigations and attempts to retrieve. Additionally smell was the only bait characteristics that influenced bait uptake in populations of badger naïve to supplementary feeding. A preference for taste was not consistent, with sweet and un-sweet being taken more often depending on social and environmental conditions. Season was found to influence bait disappearance and associated behaviours, and autumn appears the most favorable season to deploy an oral bait to badgers. Bait characteristics and deployment considerations that will aid the highest uptake of bait by badgers are proposed.

Introduction

Bovine Tuberculosis (bTB) in cattle is one of the UK's most serious animal health problems and has harmful economic implications (House of Commons, 2008). The European badger (*Meles meles*) is susceptible to bTB and is widely believed to constitute the most important wildlife reservoir of this disease (Delahay *et al.* 2002). There is substantial evidence to suggest that transmission occurs between badgers and cattle (Gavier-Widen *et al.* 2001; Philips *et al.* 2003; McDonald *et al.*2008) however the precise mechanisms remain unknown (House of Commons, 2008; Tolhurst *et al*. 2009). With the number of cattle infected with bTB doubling every four and a half years (House of Commons, 2008) implementation of effective measures to reduce the transmission risk of this disease between badgers and cattle in urgently required. In an attempt to control the disease several strategies of badger removal have been implemented since the 1970s. The Randomised Badger Culling Trial, undertaken from 1998 to 2006, reported both reductions and increases in incidence of bTB in cattle herds (ISG, 2007). The results suggest that social perturbation, as a result of culling, may potentially increase the transmission of bTB through disrupted territoriality, increased migration and mixing between social groups (Carter *et al.* 2007). These effects have been

proposed as an explanation for the failure of culling to demonstrate consistent reductions of bTB in cattle (Macdonald *et al*. 2006; Woodroffe *et al.* 2006). The mixed positive and negative effects of culling and its highly controversial nature has resulted in recent research switching focus to vaccination of badgers as a long term strategy to manage the disease (Southey *et al.* 2001; Delahay *et* al. 2003; Cagnacci *et* al. 2007). Recent work has demonstrated that Bacillus Calmette-Guérin (BCG) vaccination of captive badgers reduced the progression, severity and excretion of *Mycobacterium bovis* after experimental challenge (Corner *et* al., 2008; Lesellier *et al*., 2011) and currently the Badger Vaccination Deployment Project (BVDP) is underway to investigate the feasibility of direct intramuscular vaccination for badgers. However trapping and vaccinating badgers is highly labour intensive, may require ultimately prohibitive expenses (Delahay *et al*., 2009; Hughes *et al.*1996) and, in the case of rabies vaccination of dogs in the Philippines where a large segment of the population could not be trapped, intramuscular vaccination coverage has been too low to interrupt the chain of infection (Estrada *et* al., 2001).

The use of baits for oral vaccination is widely viewed as a more cost effective and sustainable method of tackling the disease (Delahay *et* al., 2003; Wilkinson *et al.*, 2004), and Aldwell *et al*. (2003a) conclude that oral baits will be the most practical and cost effective method of BCG vaccine delivery to brushtail possums in New Zealand. Additionally oral vaccination may overcome some of the acknowledged problems of trapping; such as biased capture of certain age classes and unfavorable immune responses to the stress of capture (Hughes *et al.*1996).Baits, traditionally used in the control of pest species, are increasingly used to deliver orally administered vaccines to wildlife (Steelman *et.* al. 1998; Estrada *et al.,* 2001).Wide scale oral rabies vaccination programmes in Europe have coincided with dramatic reductions of rabies incidence in the red fox (*Vulpes vulpes*), the main target species (Cross, *et al.,* 2007) with successful control of the disease even with vaccine uptake rates as low as 50% (Wandeler *et al*. 1988).

Much research has focused on determining the most palatable and therefore well accepted bait for target populations. In many studies of bait preferences, animals have shown strong significant preferences for particular baits, for example red foxes preferentially ate baits made of beef and honey (Suander & Harris, 2000) and African wild dogs showed significant preference for chicken heads over other bait types(Knobel *et al.,* 2002). It is acknowledged that vaccination campaigns should be adapted for each target species due to differences in food preferences and foraging habitats and much research documents that although badgers appear to specialise on earthworms, their diets can vary greatly (Kruuk & Parish, 1981), providing opportunities to investigate a range of potentially palatable types of bait (Palphramand *et al.* 2011). Cagnacci *et al.* (2007) trialed several bait types similar to food items found naturally by badgers. They document meat and fruit baits

were taken significantly more than cereal baits. Cagnacci & Massei (2008) also reported high removal of minced beef baits by badgers. However due to UK legislation (Animal By-products Regulations 2005) which prohibits bringing animal by-products on to any premises where livestock are kept, baits used to vaccinate badgers could not contain any animal derived products. Therefore establishing a meat-free bait that still results in high consumption by badgers is now necessary. Different tasting baits are often the focus of bait preference studies. Sweet baits are known to be attractive to rodent species (Marsh, 1988) and work into oral rabies vaccines for Gray foxes showed high preferences for sweet marshmallow baits and baits coated in sugar (Steelman *et al*. 1998). Southhey *et al.* (2001) have shown high uptake of sweetened baits by badgers, however reported wide variations in the proportion of badgers consuming baits (from 20% - 80%). The authors suggest that this variation was potentially due to the season of bait deployment, the availability of other natural food sources and the population densities of target and non target species.

Odorous compounds have long been used to draw animals to control devices such as baits and traps (Turkowski et al 1983; Campbell & Long, 2008) and olfactory cues may enhance bait discovery especially for those species that utilise this sense during foraging. Work developing baits for red foxes trialed baits containing olfactory attractants mimicing food, such as synthetic fermented egg, with some success. They also incorporated attractant chemicals from the anal sac secretions of foxes, to try and take advantage of the fox's scent-marking behaviour, however results were variable, and gustatory additives, such as beef flavouring, had a greater affect on bait acceptance (Saunders & Harris, 2000). Badgers are crepuscular in nature, being active at dawn and dusk. Foraging in low light conditions suggests olfactory cues may play an important role in food, and therefore bait discovery.

Problems often reported from bait trials are potential bait competition and monoplistion by a few dominant individuals, as reported in wild African dogs (Knobel *et al*., 2002) and suggested to occur in badgers (Cagnacci & Massei, 2008), and problems with the eating behviour that different baits elicit. Oral vaccination has two main potential routes of vaccination, through mucosal membranes in the oral cavity and membranes in the gastro-intestinal tract. A bait that stimulates a lot of chewing, and therefore rupturing the incorporated vaccine capsule, will allow vaccine absorption in the oralpharageal region (Wlodkowski & Linhart, 1998). Baits which elicit little chewing behaviour and allows the vaccine capsule to pass whole into the digestive tract will, if sufficiently protected from gastric secretions, allow transfer of the vaccine through the intestinal epithelium (Aldwell *et al*., 2003b). If the desirable route of vaccination is known then analysing the handling of bait by animals is crucial. For example, of the two baits favoured by gray foxes during trials, only the marshmallow wax cakes stuck to the teeth of the foxes and caused them to tip their heads back, ensuring the

retention of the liquid in the mouth and not drip out , increasing the chance of successful vaccination. For the vaccination of badgers with BCG the route of delivery will most likely be decided on the chewing behaviour that the most palatable bait elicits, therefore collecting information of how badgers handle bait is critical. Additionally the time it takes to consume a bait may determine how many individuals can access baits, affecting the ability of bait monoplisation by a few individuals. The method of deploying baits will also be a critical factor in a successful vaccination campaign. For example Palphramand *et al.* (2011) although reporting high levels of bait disappearance by badgers in both spring and summer (99% and 100% respectively), the use of biomarkers revealed that cubs only took bait in the summer .

Bait acceptance trials are essential to understand factors affecting bait disappearance by badgers and asses the feasibility for an oral vaccine campaign. The aims of this study were to understand the influence that bait characteristics, sett factors and other factors; such as season, and meteorological conditions, have on the bait disappearance by badgers and non-target species and the behaviour show towards baits, particularly preference behviour. Most previous studies have investigated bait acceptance rates in populations of badger habituated to supplementary feeding (Baker *et al.,* 2005; Cagnacci *et al.,* 2005; Cagnacci *et* al., 2007). In addition this study investigates the reaction of a population of badgers naïve to supplementary feeding, a crucial factor in the potential success of a widespread vaccination campaign.

Methods

Study areas
The experiment was conducted on four badger social groups in the 11km² study area of Woodchester Park, Gloucestershire, UK (51°43'N, 2°16'E). Three studies were conducted; Study 1(29th October – 7th November 2008), Study 2 (25th August – 3rd September 2009) and Study 3 (27th October – 5th November 2009). Badger population density at Woodchester Park is high (28adults/km²) and badgers are used to regular feeding during bait marking studies and trapping events. Baits were deployed only at the main sett in each of the social group territories. Additionally four badger social groups in an approximately 17 km² study area near Tortworth, Gloucestershire, UK (51°38'N, 2°25W) were investigated (Study 4: 8th – 17th June 2010). These previously unstudied social groups were investigated to identify how badgers 'naive' to supplementary feeding reacted to oral baits.

Experimental design

For study 1, 24 baits were deployed at each sett per day, for 10 consecutive nights; for studies 2 and 3, 32 baits were deployed at each sett per day, for 10 consecutive nights (Figure 1). 14 different baits (names withheld for commercial confidentiality reasons) were trialled. All baits bar one contained some form of peanut (either crushed or oil) and contained varying amounts of sweeteners and binding agents. Baits varied in characteristics : 'smell', 'taste', 'size' and 'composition'. For 'smell' and 'taste' baits were ranked from 1-4 for each characteristic and then assigned a status: Smelly: Un-smelly and Sweet: Un-sweet depending on their rank value. Bait size was categorised into Small, Medium or Large depending on their relative size. Three types of bait composition were identified; Hard baits, Hard + chewy baits and Soft baits. Different combinations of baits with different combinations of these bait characteristics were trialled in each study (Fig. 1). The design of the trials and the combinations of bait characteristics changed throughout as the baits were selected from previous trials and developed. Hence this study is no fully orthogonal.

Baits were placed in a depression in the ground and covered by small paving slabs (30cm x 30cm, ~ 5kg), to protect them from the weather and non-target species . The slabs were positioned randomly around each sett and baits were deployed the afternoon before the study night.

Data collection

Bait disappearance was measured on daily bait disappearance rates. The number of baits taken by badgers, eaten by non-target species, or remaining were recorded each morning following a study night. Non target bait disappearance was inferred from obvious tunnelling under slabs and tooth marks on bait remains. All baits were replenished/replaced daily for the duration of the study. Observational data recorded by a remote video camera was used to measure behaviour towards baits. The field view contained a subset of the baits being trialled (Study 1 = 3 slabs, Studies 2,3 &4 = 4 slabs, placed approximately 1m apart). Under infra-red illumination animals could participate in the study in total darkness. The spatial arrangement of baits was rotated every other day to ensure that factors such as direction of approach and learned location did not bias results. Each camera was set to record for 12 hours per night, starting prior to expected emergence times and lasting for the duration of the night's activity. The compact flash memory cards (16gb) in each camera, were collected and replaced daily. Video surveillance was conducted on 40 nights at each main sett per study (i.e. for ten nights at each of the four setts), with a total of 120 nights of surveillance across studies 1, 2 and 3 and 40 nights for study 4.

Social group territory size for studies 1, 2 & 3 was estimated using a Minimum Convex Polygon method ((MCP; see Hayne, 1949; Delahay *et al*., 2000) using data obtained from yearly bait marking studies and observational field signs (see Delahay *et.al*. 2000) and ranged from 0.06km² to 0.42km².

The amount of different types of habitat available to badgers within a social group was estimated by analysing aerial maps in Geographical Information Systems (GIS) analysis. Social group MCPs were overlaid onto aerial photographs (Scale) and areas of different habitat types calculated. As social groups territories are dynamic habitat estimates were calculated for each year. As the precise date when the aerial photographs were taken was unknown and the changeable use of land for arable or pasture practices, these habitat types were grouped into one category (AP). Area of woodland (W) was also calculated. For study 4 bait marking studies were not undertaken as this would give prior exposure to being fed a bait. Social group habitat size was therefore delineated as a circle with a 500m radius from the main sett, encompassing an area slightly smaller than 1km². This area was selected as it is similar to the largest reported territory in the south of England (Cheesman *et al.* 1989).

The population size of each social group in studies 1, 2 & 3 was estimated using Minimum number alive (MNA) indices using capture-mark-recapture data. MNA is defined as the number of individuals caught in a capture session, plus those that were not caught at that time but were caught both previously and subsequently (Krebs, 1966). Values of the minimum number of cubs alive were also estimated. MNA indices were calculated for each year, therefore each social group in each study had total MNA estimate and a cub MNA estimate assigned to it. Population size could not be estimated for social groups in study 4 as trapping did not take place.

The studies were undertaken in two seasons, defined as; summer: 21_{st} June- 21_{st} September; autumn: 22nd September- 1_{st} December (BBC, 2000). Microclimatic conditions are known to affect the surfacing of worms and in turn the availability of natural food for badgers (Kruuk & Parish, 1981) therefore data on meteorological conditions; rain and temperature were collected from local weather stations (Studies 1, 2 & 3: Nailsworth weather station UK; Study 4: Dursley Highfields UK). Rain was measured as the total amount of rain over a 24 hour period from 7:00am the morning before a study night until 7:00am the following morning. This time period was chosen as rain before emergence may affect earthworm availability and also behaviour during badger activity. Temperature was measured as the average temperature from one hour before sunset until sunrise. As temperature did not vary during study 4 it was not included in analysis.

Statistical analysis

Differences in badger and non target bait disappearance between habituated and naive badgers were tested separately. Bait disappearance was entered as the dependent variable in a GLMM, to identify variation associated with explanatory variables; 1) Bait characteristics; smell, taste, size and composition, 2) Sett factors; total animals MNA estimates, cub MNA estimates, territory size, amount of arable/pasture and woodland in territory, and 3) Other factors; day of study, season,

temperature, rainfall. Bait characteristics were analysed independently not in combination (i.e. didn't analyses the affect of sweet smelly large baits against sweet smelly small baits) as not all combinations existed, however some basic descriptive statistics were carried out on combinations of smell and taste.

Using video surveillance data five main factors of interest were analysed: (1) Investigation: the number of investigations (sniffing) towards baits (2) Attempt to retrieve: whether there was an attempt to move the slab to retrieve the bait (3) Preference: whether the bait was eaten as encountered or preferred, after at least one bait had been investigated, (4) Rejection: whether once an attempt to retrieve a bait was made it was pursued until eaten or rejected and (5) Time to consume a bait. For video footage data to be included in the analysis at least one bait had to be eaten from the subset available (i.e. investigations or attempts without subsequent bait retrieval were not included). For these 'Events' when at least one bait was taken, it was recorded which baits stimulated the five behaviours of interest and which did not. The video footage analysis only included 'first encounter' data; information collected when the full selection of baits was available to the individual (i.e. it excluded footage where one or more baits had already been taken and therefore a full choice was not available). Including only this 'first encounter' data allowed anaylsis of preference behaviour. Investigation, Attempt to retrieve, Preference, Rejection and Time to consume were entered as dependent variables in separate GLMMs with the same explanatory variables as before. Analyses were performed in R (v 2.6.2). Analysis was not carried out on behviour towards baits captured by video surveillance in study 4 (naïve), as sample sizes were too small, with baits observed being eaten on only seven occasions.

It must be noted that statistical analysis of differences between the habituated and naïve study groups were not carried out, as only two sites were investigated, therefore any apparent differences discussed between the two groups may be a result of naivety to being fed baits, but also could be explained by site specific differences.

Results

Bait Disappearance

Studies 1, 2 & 3 - Habituated

Of the 3520 baits deployed during the habituated studies, 2776 (79%) were taken by badgers. Day (χ^2_1=60.1, P<0.001), season (χ^2_1=12.5, P<0.001) and the amount of arable and/or pasture in each social groups territory (χ^2_1=15.4, P<0.001) were identified as statistically significant predictors of

bait disappearance. Bait disappearance increased over the duration of each 10 day study (Fig 2), with disappearance higher in autumn (89%) than summer(61%). Social groups whose territories contained higher areas of arable and pasture habitat had increased bait disappearance. An interaction between the bait characteristic 'taste' and a social group's territory size was also found be significant (χ^2_1=14.5, P<0.001). With increasing territory size the number of baits taken decreased, however un-sweet baits less so than sweet. Interactions between bait characteristic 'taste' and the total MNA and cub MNA estimates had a significant effect on bait disappearance ((χ^2_1=7.13, P= 0.008). As the total number of animals in a social group and the number of cubs within a group increased bait disappearance increased, with disappearance of sweet baits increasing more than un-sweet.

Neither rainfall, temperature nor bait characteristic 'smell' were significantly correlated with the number of baits taken.

Study 4 - Naïve

Of the 1280 baits deployed during the naïve study, 87 (6.8%) were taken by badgers. An interaction between bait characteristic 'smell' and day had a significant affect on bait disappearance (χ^2_1=7.3, P= 0.007). The amount of Arable and Pasture in each social groups predicted territory (χ^2_1=15.4, P<0.001) was identified as statistically significant predictors of bait disappearance. Over the duration of the study more baits disappeared with smelly baits taken more often (Fig 3b). Social groups whose territories contained higher areas of arable and pasture habitat had lower bait disappearances. Bait characteristic 'taste' had no significant effect on the disappearance of baits.

Behaviour

Studies 1, 2 & 3 – Habituated

Of the 120 nights on which video surveillance was conducted across the habituated studies, footage was obtained on 109 of those nights and badgers were observed on 103 of these nights. 480 baits were available under video surveillance across setts over studies 1, 2 &3. Of these 90 were observed being taken first.

Investigation

703 investigations towards baits were recorded from video surveillance. Day of study (χ^2_1=7.2, P=0.006) and Season (χ^2_1=6.5, P<0.01) had a statistically significant effect on the number of investigations towards baits. Bait characteristic 'smell' (χ^2_1=4.9, P=0.02) significantly affected the number of investigations a bait received. The number of investigations towards baits per night

increased over the duration of each 10 day study with investigations more numerous in autumn than summer. Smelly baits received significantly more investigations then un-smelly baits. As the estimated total number of animals in a social group increased the number of investigations increased significantly (χ^2_1=8.65, P=0.003)

Attempts to retrieve baits

Attempts were made to move slabs on 172 occasions. Day of study (χ^2_1=35.84, P<0.001), Season (χ^2_1=7.45, P=0.006) and social group territory size (χ^2_1=5.7, P=0.02) were all statistically significant factors affecting whether attempts were made to access baits from under bait slabs. Bait characteristic 'smell' (χ^2_1=4.1, P<0.001) was a highly statistically significant predictor of which baits would elicit an attempt to access. More attempts to access baits were made in autumn than summer and as territory size increased significantly fewer attempts were made to access baits. A greater amount of attempts were made to access smelly than un-smelly baits. As the estimated total number of animals in a social group and the number of cubs within a group increased the number of attempts to access baits increased significantly (Total animals: χ^2_1=4.08, P=0.04, Cubs: χ^2_1=6.51, P=0.01).

Preference

Of the 90 baits observed being taken first by badgers 29 (32%) were as encountered and 61 (68%) preferentially, after investigating at least one other bait. Preference behaviour was significantly affected by territory size (χ^2_1=9.5, P=0.002) and bait size (χ^2_1=8.6, P=0.01). As a social group territory size increased less baits were eaten as encountered and more baits were eaten preferentially.The effect of size of bait on preference behaviour differed significantly between baits with only large baits resulting in a difference in the number eaten as encountered or preferentially (Fig. 4). Bait characteristics smell and taste did not act as a significant predictor of preference behavior.

Rejection

Of the 166 attempts made to access baits, 23 (14%) were subsequently rejected and no longer pursued by that animal. Bait characteristic 'smell' (χ^2_1=43.3, P<0.001) and bait size (χ^2_2=22.5, P<0.001) significantly affected bait rejection. Significantly more un-smelly baits (83%) were rejected than smelly baits (4%). The percentage of bait rejection differed significantly across all three bait sizes, with small baits accounting for significantly more bait rejection(18%) than medium (15%) or

large (12%) sized baits. Bait characteristic taste did not significantly influence whether a bait was rejected or not.

Time to consume baits

Bait size (χ_{22}=172.4, P<0.001) and bait composition (χ_{22}=12.5, P=0.002) were statically significant predictors of the time taken to consume a bait. On average soft baits took 12 (±5.4 SE) seconds to consume, which was significantly faster than hard and chewy baits (43 secs ± 3.3) and hard baits (53 secs ± 6.5). On average small baits took 5 seconds(± 1) to consume, which was significantly different from medium baits (56 secs ± 5.4). Large baits took on average 43 seconds (± 3.2) to consume.

Non target bait disappearance

Studies 1, 2 & 3 – Habituated

Of the 3520 baits deployed during study 1, 2 and 3, 129 (4.7%) were taken by non target species; mainly rodents and invertebrates (identified from field signs). Day of study (χ_{21}=21.5, P<0.001) and temperature (χ_{21}=9.5, P= 0.002) had a significant effect on the number of baits taken by non target species. Bait characteristics taste (χ_{21}=10.1, P=0.002) was also found to be significant. As the studies progressed (Fig. 2) and on nights when temperatures were lower, non-target consumption of baits decreased. Non target species also showed a preference for sweet baits over un-sweet baits.

Study 4 – Naive

Of the 1280 baits deployed during study 4, non targets consumed 225(17.5%) of these. Bait characteristics smell (χ_{21}=7.51, P<0.001) and taste (χ_{21}=7.51, P<0.001) significantly affected bait disappearance. Smelly baits and sweet baits were taken in significantly higher quantities than un-smelly and un-sweet baits respectively.

Discussion

Badger Bait disappearance and handling behviour

The results from video surveillance in this study confirm previous findings that badgers exhibit preference behviour towards baits, with 68% of baits available being eaten preferentially after investigating at least one other bait, and only 32% of baits being eaten as encountered. This study also demonstrated that bait characteristics have a significant effect on bait disappearance and preference behviour towards baits. Although the flavour of baits is often targeted for manipulation it

appeared from this study that the smell was of greater importance than taste. Smelly baits resulted in increased behaviour- attracting more investigations and accessing behaviour and less bait rejection. Additionally smell was the only bait characteristics which had a significant effect on bait disappearance in the naive population. The fact that smell was not a significant predictor of bait disappearance in the habituated population is likely a result of overall high disappearance rates, so after smelly baits were preferentially consumed, most other baits were then subsequently eaten. Smell is often the primary sense used by foraging animals, especially in low light condition where visual abilities are reduced. Naive badgers presented with novel food items are likely to have to utilise olfactory senses more than habituated badgers that know baits are often present on or around the sett. This indicates that, as badgers are known to follow conspicuous paths away from setts to natural feeding areas, and can travel relatively fast sometimes only picking up odd food items on the way (Kruuk, 1978), baits used for a widespread campaign would have to contain strong olfactory cues. Several studies have tested baits containing olfactory attractants and demonstrated them to enhance bait discovery (i.e. Linhart *et al.*, 1994). Campbell & Long (2008) demonstrated that feral swine showed increased investigatory behviour towards stations emitting apple and strawberry scents (cotton wool soaked in liquid feed additives), than to control stations with no olfactory attractant. The authors suggest the incorporation of such additives into oral delivery systems, such as vaccines, for feral swine, and noted the species specific application of an attractant may be of paramount importance.

Bait characteristic taste, although having a significant affect on bait disappearance when interacting with sett factors, did not show a consistency of one taste to increase bait preference. Sweet baits were eaten more often when the estimated total number of animals at a sett increased however as territory size of a social group increased, and the number of baits being eaten decreased, sweet baits were eaten less than un-sweet. Variation in preference for sweet or un-sweet baits (Fig. 3a) may be explained by potential differences in the current nutritional status of animals, which has been shown to influence food preference; for example glucose and sucrose taste pleasant to fasted humans, but become unpleasant after they are consumed (Cabanac, 1971). Mammals have evolved to make strong associations particularly with flavour and/or smell of foods and animals have demonstrated the ability to closely correlate the nutritional value of foods with smell, taste or appearance characteristics (Forbes & Kyriazakis, 1995). Ashely (1985) demonstrated that laboratory animals are prepared to work harder for some nutrients (such as protein and minerals) than others (such as carbohydrates and fats) and this may explain differences in the rejection rates of smelly and un-smelly baits, with smelly baits stimulating continued effort to retrieve which badgers could potentially asses as being of greater nutritional value. Additionally as badgers in our studies showed

significant preference behviour only towards large baits, this could be related to requiring a significant reward for effort spend accessing a bait, or that such baits had a larger surface area and potentially emitted a stronger olfactory signal.

Nutritional requirements of animals change over time i.e. from juveniles to adults. It is known that female badgers rely heavily on stored fat during gestation and the initial stage of lactation (Domingo-Roura *et al*. 2001) and therefore using this knowledge to provide a time specific high fat content bait may increase uptake rates. Future studies trialing the most palatable and preferred bait to cubs may allow a targeted baited system towards this age cohort immediately after cub emergence from setts. It is also worth noting that experiences early on in life are known to affect future behaviors. For example familiarity with foods that a mother eats just before and after birth can be translated to young through the mother's milk. Additionally interactions with mother and peers, combined with the consequences of food ingestion, help young animals to discriminate among foods and influence preferences and foraging behaviour (Provenza *et al.,* 1998). Therefore there is an argument for a period of pre-baiting with vaccine-free baits, just before the emergence of cubs from the sett, when they are still receiving milk from and learning foraging behaviors from their mothers. This may result in a quicker uptake in baits containing vaccine, by cubs soon after emergence; a highly desirable outcome. Preference of a food item is dependent on palatability; defined as a functional integration of senses (smell, taste and texture) and post-ingestive feedbacks (effects of nutrients on chemo-, osmo- and mechano-receptors), as influenced by an animals physiological condition and a foods chemical characteristic (Provenza *et al*. 1998). Our current study did analyses significant difference of baits with different combinations of smells and tastes, for example some smelly baits were sweet and other un-sweet (However see table 1 for descriptive statistics). Future bait trials may wish to investigate which combination of smells and tastes are most preferred by badgers.

The significant differences in bait disappearance by social groups could be attributed to differences in territory size and the amount of arable and/or pasture in each social group's territory. Those social groups with larger territories showed reduced attempts to move slabs to access baits and reduced rates of bait disappearance than those with smaller territories. Larger territories may provide access to a greater quantity of natural food resources and Fletcher *et al.* (1990), reported low bait acceptance by raccoons was likely influenced by the availability of ripe wild grapes at the time of the study. As a social groups territory size increased less baits were eaten as encountered and more baits were eaten preferentially. With larger territories potentially providing more food badgers may be able to be choosier. When investigating bait uptake by urban foxes, Trewhella *et al.*(1991) inferred that food was not a limiting resource to these urban populations, and the

availability of a variety of food types could be negatively influencing bait uptake. This highlights that where other food is abundant a bait needs to elicit much stronger cues to be competitive with regularly available alternatives (Saunders *et. al.* 2000). Baits may have greater uptake in those social groups with smaller territories but contain higher densities of animals, as here competition between animals should be higher. This is demonstrated by sett A which had the smallest territory size (0.14km² (study 1), 0.06km² (study 2)) but the highest minimum number alive estimate (16 MNA (study 1), 15 MNA (study2)), and also exhibited the least preferential behaviour eating 46% of baits as encountered. The practical implications of these results would be a potentially reduced period of bait deployment for social groups with known smaller territories and/or high number of individuals within each territory, reducing the costs of bait delivery. Tailoring bait densities according to target species densities has been suggested by many authors (Hable *et al.,* 1992; Blackwell *et. al.,* 2004). Many studies have reported the earthworm *Lumbricus terrestris*, as the badgers principle prey (Kruuk & Parish, 1985; da Silva *et al.,* 1993). Differing habitat types are known to contain different densities of earthworms. Earthworms are known to be most abundant in pasture (Kruuk *et al*. 1979) and therefore it could be concluded that those social groups whose territories contain higher levels of pasture would consume less baits as natural resources are higher, and this was found to be true for our naive study. However the results of our habituated study were contrary to this. Those animals with the greatest areas of arable/pasture showed greater investigatory behaviour towards bait and higher disappearance rates. Suitable microclimate conditions (high humidity and low wind speed) stimulate surfacing behaviour of worms, and therefore availability to badgers. Consequently the presence of worm dense pasture may be unprofitable if conditions are not favourable. Territories with more woodland than arable and pasture, may provide greater food security for badgers as woodland tends to provide a wider variety of foods in addition to invertebrates (e.g. berries, nuts and carrion) and through vegetation cover may maintain suitable microclimatic conditions for surfacing worms (Kruuk & Parish, 1981). The amount of alternative food sources available to badgers when baits are deployed may be a crucial factor determining uptake and Trewhella et al (1991)documented considerable variation in the proportion of baits taken form the different habitat types, by foxes, suggesting differences in resource availability dependent on habitat type. When nutritional and physiological conditions are adequate, it has been demonstrated that animals eat small amounts of novel foods (i.e. they are neophobic). However, the fact that for this study pasture was combined with arable land may confound results. Arable land is known to have low densities of earthworms, pasture has been shown to contain twice the density of worms than arable (Silva *et al.*, 1993), and is only profitable to badgers at certain times of the year when crops are available (Kruuk & Parish, 1985). From the results of our study it can be concluded that using

basic and grouped estimates of habitat as a proxy for available natural food resources is not a reliable indicator for predicting bait disappearance. A more detailed and current estimate of actual food availability in each territory would be required to determine if natural food abundance significantly influences bait disappearance.

As the estimated number of badgers in a social group increased investigatory behaviour towards baits and attempts to retrieve baits increased as did bait disappearance. Larger groups of animals are likely to have greater levels of intraspecific competition, especially if natural resources are low, and therefore may take baits more readily. It is also hypothesized that one advantage of group living is the opportunity to share information and learn from other group members (Clark & Mangel, 1986). Groups of animals can act as "information centres", where individual animals can enhance their feeding success by following more successful animals to sources of food, for example terns that returned to the colony with a fish, were more likely to be followed on subsequent trips (Waltz, 1987). Studies by Kruuk (1978) on natural foraging reported that badgers were only seen foraging in groups very rarely and that it was unlikely that badgers gained foraging experience by learning from each other. However learning behaviour has not been investigated for manmade baits disturbed on or around setts, and could explain the increased disappearance of baits in groups with more individuals. It must be noted that the results could also just be a product of more animals resulting in a greater bait encounter probability.

There was an increase in (i) bait disappearance (Fig. 2), (ii) the number of investigations and (iii) the number of attempts made to access baits as each study progressed. This is similar to results from bait disappearance studies with urban foxes which showed as steep increase over the first 2-3 days before approaching an asymptote (Trewhella *et al.*, 1991). This behviour is suggestive of a neophobic response to novel food items. Knoble *et al.* (2002), trialing oral rabies baits for free-ranging African wild dogs (*Lycaon pictus*), noted that subordinate African wild dogs often displayed such behviour, often being seen investigating baits but not ingesting them. They also went on to report increased pack coverage with increased exposure of the pack to baits, noting how once a dog ingested a bait it would seldom ignore further baits and would appear to actively search them out. Additionally the baits trialed during our studies contained ingredients quite different of food types badgers would find naturally (i.e. contained peanuts, vanilla extract, sweeteners etc) and investigations of sheep and goat grazing behviour reported that these species typically avoid flavor concentrations that are most different from what they have eaten in the past (Provenza *et al*. 1994).

For habituated social groups 52% of the baits available were taken on day one, which increased to 74% on day five and 91% on day 10. In contrast, only 6.8% of baits were taken by naïve badgers in study 4 over the duration of the 10 day study. The potential implications of 93% of baits being left in

the field includes large losses to non target species and reduced cost effectiveness (Saunders *et al.*, 1999). The increase in behavioral responses and bait disappearance over time, coupled with the low disappearance by naive badgers noted here highlights the need for a pre-baiting period where baits without vaccine are deployed. Only when bait disappearance is sufficiently high should baits containing the vaccine be deployed. Measuring disappearance rates of baits can be a useful way of measuring the numbers of baits being taken and preferences of a social group, however high disappearance rates do not necessarily translate into high proportions of badgers within a social group eating the bait (termed bait 'uptake'). Cagnacci *et al.* (2007) who incorporated biomarkers into their baits, reported that although disappearance rates of meat baits were higher than cereal baits, a smaller proportion of badgers appeared to consume these meat baits.

The optimum time to deploy baits if often believed to be dependent on season and its associated meteorological conditions and natural food availability (REFs). A study into bait dissapperence by urabn foxes reported a steady increase in the number baits investigated or taken throughout the year from 27% in January/February to 56% in September/October (Trewhella *et al.*, 1991) The reduced quantity of natural food in summer, particularly the scarcity of worms, suggests that this time of year would be most appropriate for delivering baits to badgers. This is supported by recent work by Palphramand *et al.* (2011) who reported higher uptake rates of peanut and syrup bait in summer than in spring. However our results report a significant increase in investigatory and retrieval behaviour and bait disappearance in autumn compared to summer. Badgers exhibit seasonal weight gains, increasing their body-fat levels in late summer and early autumn (Woodroffe, 1995) as an adaptation to cope with likely food scarcity in winter conditions. This may explain the increased attempts to retrieve baits in autumn, which bar one, all contained some form of fatty peanut oil. However, as vaccinating susceptible cubs against btb is crucial to the success of a vaccine campaign, ensuring cubs receive a vaccine as early as possible is essential. Palphramand *et al.* (2011) reported that 91% of cubs captured in summer showed evidence of having taken bait. If vaccine deployment was delayed until autumn, the chances of these previously susceptible cubs, having now contracted the disease will potentially increase. Mason *et al.* (1996) also found differences in bait uptake rates by foxes according to season but found repeat baiting campaigns improved bait acceptance, therefore after several years of deploying bait, the season in which they are deployed has potential to be more flexible. Additionally it is worth noting that badgers known to regurgitate food to young, therefore BCG could potentially be passed from mother to young that have not emerged from setts (Hughes *et al.,* 1996), allowing baits to deployed in spring, to coincide with increased badger activity. However a much greater understanding on the frequency of this

behaviour and whether viable vaccine could transferred via this vertical route, would be required to suggest delivering bait so early on in the year, before cub emergence.

Meteorological factors temperature and rain had little effect on disappearance of baits by badgers, however if baits were deployed in winter these conditions may have an affect.

Non target bait disappearance

Non target species in both study areas (Woodchester park and Tortworth) showed a preference for sweet baits over un-sweet baits and in results form study 4 indicate smelly baits were preferred. Sweet baits (e.g. white chocolate) have been used for pest control operations, and Beath *et al*. (2004) reported that short tailed bats *(Mystacina tuberculata)* were attracted to smelly cinnamon baits. As studies 1,2 & 3 (habituated) progressed, bait consumption by non-targets decreased (Fig. 2). This correlated with a significant increase in bait disappearance by badgers (Fig. 2), and therefore suggests that non targets do not restrict the numbers of baits available to badgers, and will consume what is left. For study 4 non target bait disappearance as the study progressed did not decrease as in the other studies, but fluctuated around 15% disappearance (Fig.2). This is likely because bait consumptions by badgers was low during the duration of the study and therefore many baits were available. Although temperature did not affect badger bait disappearances higher temperatures increased the disappearance of baits by non targets, explained by greater activity of rodents and invertebrates in warmer weather (Vickery & Bider, 1981).

Recommendations

The Bait

From the results of this study I would recommend a smelly, un-sweet bait be selected to deliver an oral bcg vaccine. Although taste did not have a consistent significant affect on bait disappearance a overall un-sweet baits were consumed more often (Table 1). As non target species ate significantly more sweet baits than un-sweet bait may result in more consumption by the target species. Although small baits resulted in less chewing, and therefore would suit the gastro-intestinal route of delivery, bait rejection of this size was significantly higher than medium or larger baits. Therefore I would suggest the oral cavity mucosal route as the most appropriate route of delivery, with large sized baits, which were the only baits to have higher preferential eating over baits eaten as encountered (Fig. 4). To support the mucosal route of delivery the most suitable composition would be hard or hard chewy baits, which took significantly longer to chew than soft baits, increasing the chance of the vaccine capsule being punctured.

Bait deployment

If the size of the social groups territory is known, through bait marking studies, then those groups with large territories may need a longer period of baiting, as they showed lower levels of bait disappearance. If the number of animals present in the sett can be estimated, then those setts with higher numbers of animals may require more baits to ensure sufficient uptake, and reduce bait monoplisation. However if vaccination is to be applied as a wide-scale approach to controlling the disease in badgers, information on territory sizes and estimates on the number of animals present, through sequential cage trapping, are unlikely to be undertaken due to expenses and logistical incapacities. Therefore bait deployers may have to rely on other proxy measures, such as prebait uptake rates and sett activity scores to ensure cost effective and efficient deployment of vaccine baits.

From this study autumn appears the optimum time to deploy baits, when investigatory and retrieval behviour towards baits is higher, as is disappearance. Also in cooler temperatures, often experienced in autumn months, non target uptake is lower, which is obviously desirable. From this study the importance of a pre bait period has been demonstrated. For those badgers habituated to supplementary feeding , bait disappearance was above 50% from day 1, but to ensure the most amount of badgers are consuming baits a pre-baiting period would be beneficial. Pre-bait is of greatest importance for badger naïve to supplementary feeding (a large proportion of the badger population in the UK). Here a pre-bait of over 10 days may be necessary, and baits should ideally only be deployed one disappearance rates are sufficient (Fig. 3b).

Additionally deployment of the baits may be tailored to ensure sufficient uptake by all members, and as noted by Cagnacci *et al.,* (2007), dispersed feeding over aggregated baits points reduce bait monopolization. Additionally incorporating chemical repellents into baits has been suggested as a method to prevent bait monopolization. Chemicals stimulating a conditioned taste aversion (CTA) (Baker *et al*., 2005), through associating the taste of a bait with an adverse post-ingestinal effect, would allow a badger to consume a sufficient number of baits to allow successful vaccination, but not to keep consuming high levels of baits. However as multiple years of oral vaccine bait uptake will be necessary to ensure sufficient vaccination of the target population, care must be taken to ensure this CTA would not still be effective during subsequent years.

The success of any vaccination campaign will be a product of the proportion of animals that receive the vaccine and the proportion that become immunised (Delahay *et al*. 2003). The ability of a oral vaccination strategy, as a single approach or in combination with culling operations, to help control bTB in the UK will be greatly affected the bait uptake by badgers. The proportion of the badger population that needs to be vaccinated to have significant effect on bTB in cattle has yet to be

determined, however vaccinating cubs before they contract the disease is central to breaking the disease transmission cycle (Wilkinson *et al*.,2004). Results from this study indicate that badgers habituated to supplementary feeding take relatively high number of baits. However future studies, using the baits and baiting strategies suggested from this work, should determine the uptake rates of such baits to ensure a sufficient proportion of cubs and adults are access the vaccine. A greater number of populations of badgers need to be studied in order to conclude that bait naivety has a significant affect on bait disappearance. Baits attractive to one social group of badgers may be less attractive to badgers in other areas, due to differences in social and environmental conditions, and therefore bait trails need to be conducted in badgers with known low populations densities (such as flat lowland areas) and also in diverse habitat types (such as urban and high moorland areas). Information on the cost of producing BCG oral baits, along with costs of delivering such baits (government or landowner led) will also greatly affect the efficacy and cost effectiveness of vaccination campaigns.

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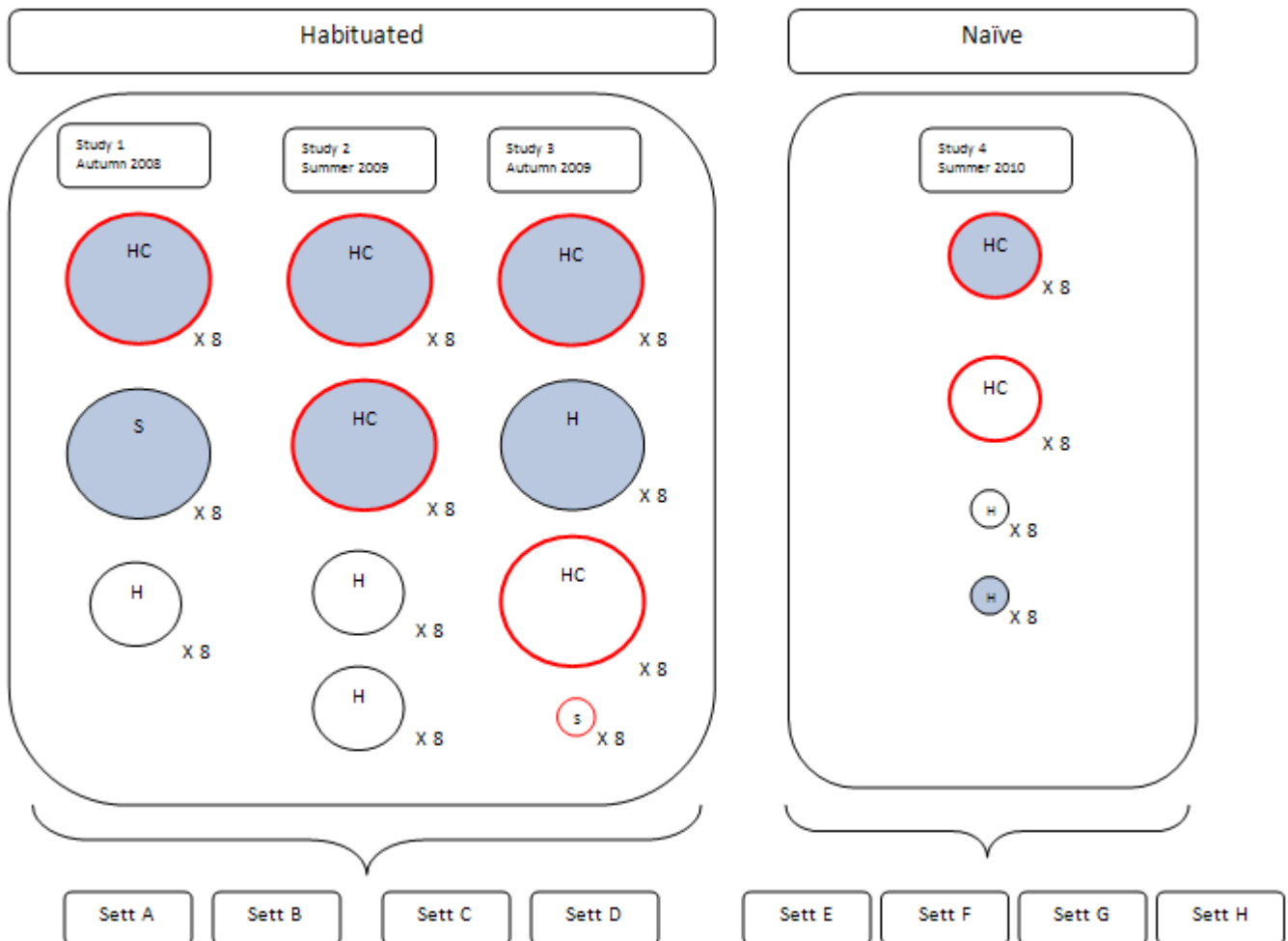
Figures and Tables

Fig. 1. Experimental design. Circles show baits fed. ⬤ Sweet, ◯ Un-sweet, ◯ Smelly, ◯ Un-smelly, HC= Hard chewy, H = Hard, S= Soft. Size of circle = Large, medium and small. X8 = 8 baits of each bait fed at each sett each night.
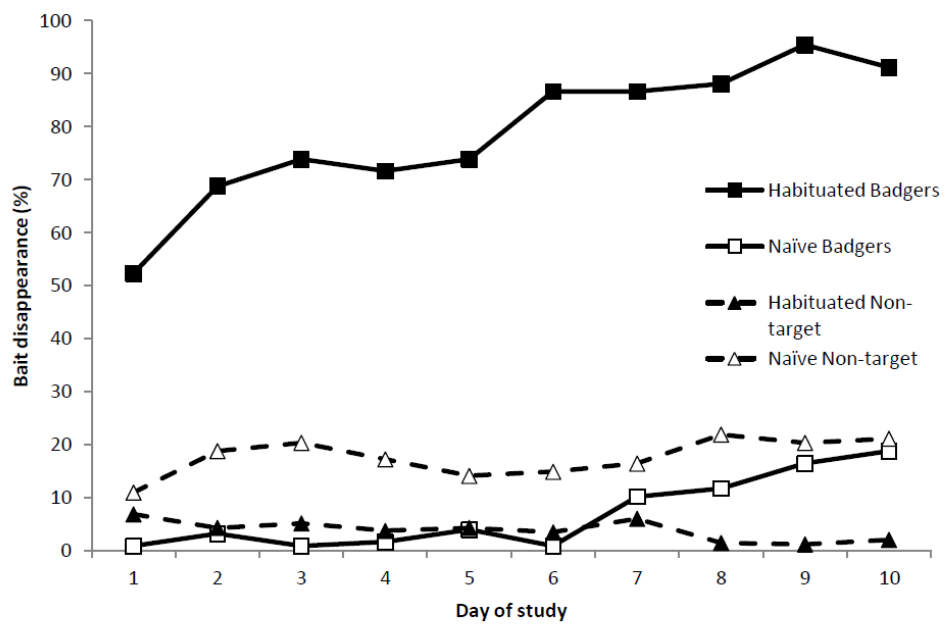
Fig. 2. The percentage of baits being taken daily by badgers and non targets, from both habituated and naïve studies.

(a) Studies 1, 2 & 3 (Habituated)

(b) Study 4 (Naïve)

Day of study

Linear (Sweet)
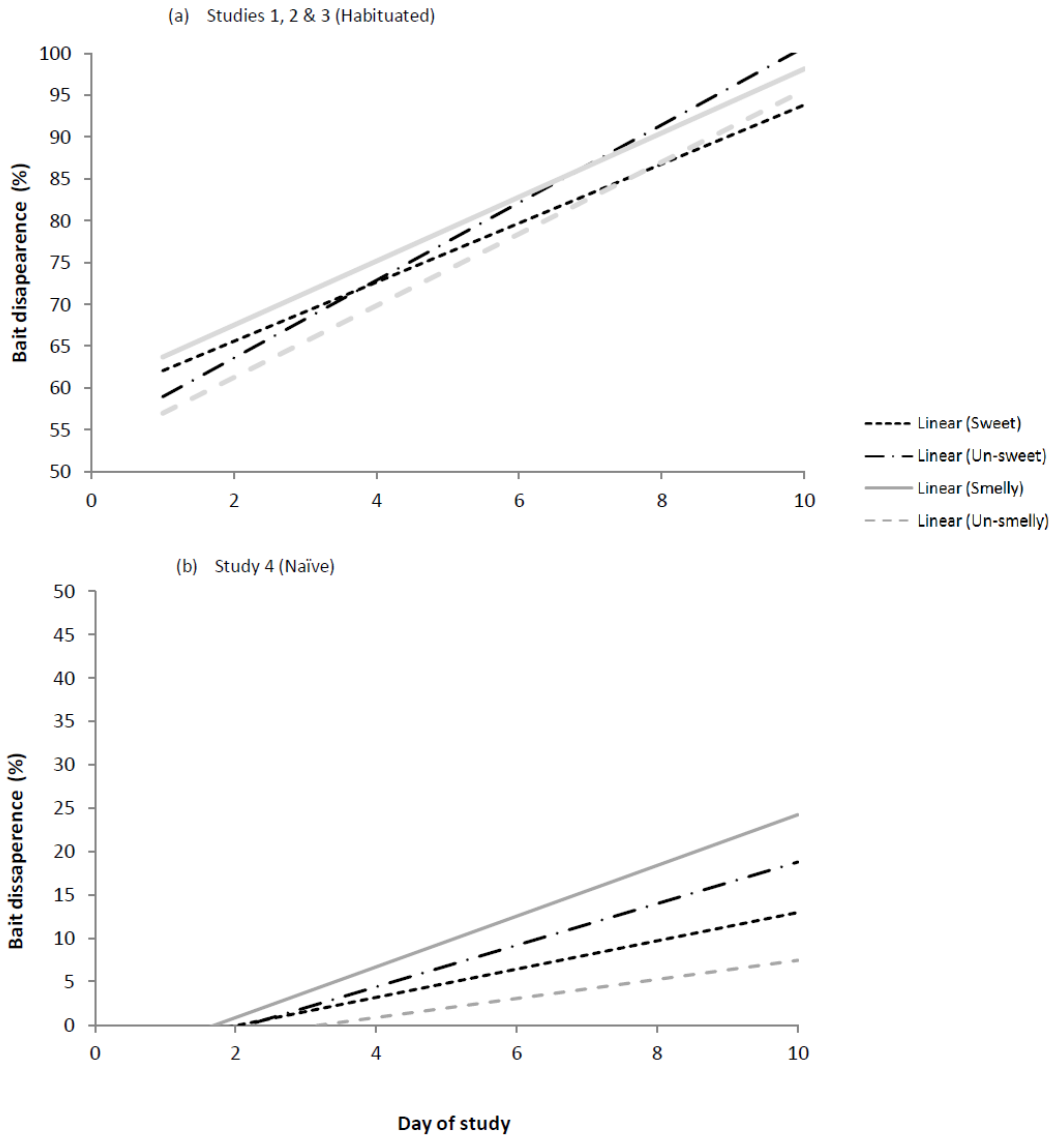Linear (Un-sweet)
Linear (Smelly)
Linear (Un-smelly)

Fig 3. The percentage of sweet, un-sweet, smelly and un-smelly baits being taken daily by badgers, from habituated (a) and naïve studies (b) (lines of best fit). Note difference on y axis: (a) 50-100%,
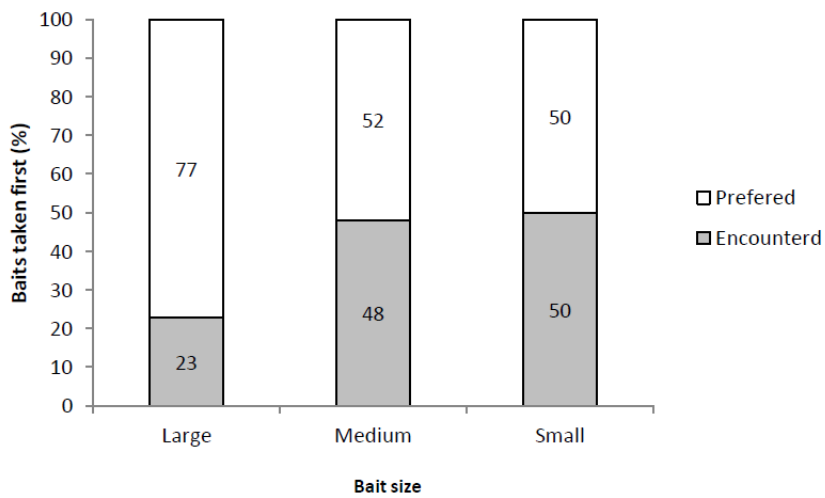
Fig. 4 The percentage of baits taken first that were encounterd or preferred by bait size .

Table 1. The total number of baits taken by badgers over studies 1, 2 & 3 (habituated) combined based on daily disappearance rates (%), separated into bait characteristics 'smell' and 'taste'. Combinations of these two characteristics are also included.

	Smelly	Un-smelly	Total
Sweet	951/1280 (74.3%)	546/640 (85.3%)	1497/1920 (78%)
Un-sweet	603/640 (94.2%)	675/960 (70.3%)	1278/1600 (80%)
Total	1554/1920 (81%)	1221/1600 (76.3%)	