The implications of bite wounding for disease status
in badgers *Meles meles*

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I certify that all material in this thesis 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.

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MRes Wildlife Disease Management

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1. INTRODUCTION

Wildlife disease comprises any impairment of normal functions in free-ranging animals. This includes both infectious and non-infectious diseases, encompassing both microparasites, such as viruses, bacteria and protozoa, and macroparasites, such as helminths and arthropods. Managing wildlife disease is a huge task growing in importance and recognition globally (Artios *et al.*, 2001; Daszak, Cunningham & Hyatt, 2000). This is clearly reflected in the upsurge of wildlife-disease related scientific papers published in peer-reviewed journals in the last two decades (Gortazar *et al.*, 2007). The majority of these focus on infectious diseases, which consequently will form the largest past of this review. Recent international meetings (such as those held by the Wildlife Disease Association) allowing veterinary officers, disease ecologists and other professionals to consult, and the formulation of multidisciplinary research teams, confirms the critical need to develop a framework for more effective management of wildlife disease, based on scientific evidence (Artois *et al.*, 2001; Gortazar *et al.*, 2007).

Whilst wild mammals have a long history of association with humans (White & Lowe, 2008), historically, diseases in wildlife have only been considered important when agriculture or human health are directly threatened (Daszak *et al.*, 2000). However, the current and potential future impacts of wildlife disease on global health, agriculture and biodiversity are becoming increasingly more widely recognised (Wobeser, 1994, 2002; Daszak *et al.*, 2000; Artios *et al.*, 2001; Delahay, Smith & Hutchings, 2009). The number of new infectious diseases emerging globally has risen significantly in the last five decades (Jones *et al.*, 2008), and wildlife epidemiological patterns are ever more closely linked with humans and domestic animals (Artios *et al.*, 2001; Daszak, Cunningham & Hyatt, 2001). Whilst disease is undoubtedly a natural component of ecosystems (Wobeser, 1994; Delahay *et al.*, 2009), there are significant correlations between the recent trend in emerging infectious diseases with human population density and wildlife species richness (Jones *et al.*, 2008). In fact, ecosystem alteration by anthropogenic activities is a major cause of emerging infectious diseases (Williams *et al.*, 2002; Jones *et al.*, 2008).

There is a clear economic cost to wildlife disease; past cases show this well. For example, the European costs of orally vaccinating red foxes against rabies totalled over US$80 million, just for baits (Stohr & Meslin, 1996), whilst in the USA, financial impacts of rabies are estimated at around $408 million annually (Sterner & Smith, 2006). Expenditure due to bovine tuberculosis cost British tax payers over £100 million in 2008-2009 (DEFRA, 2009a), and the estimated cost of the series of classical swine fever epidemics that spread from Germany across parts of Europe in 1996 was US$2.3 billion for the Netherlands alone (Meuwissen *et al.*, 1999; De Vos, Saatkamp & Huirne, 2005). The severity of future financial impacts of emerging zoonotic infectious diseases (those transmissible to humans) is difficult to predict, but likely to be considerable (Daszak *et al.*, 2000). Zoonoses dominate emerging infectious human diseases (over 60 per cent originate in wildlife) (Jones *et al.*, 2008). Wildlife disease therefore poses a significant and increasing threat.

The risk to global biodiversity and the resulting ecological and economic consequences are of similar concern (Daszak *et al.*, 2000; Gortazar *et al.*, 2007). Wildlife has great economic value, generating jobs, income, and enjoyment from viewing and learning (Macmillan & Phillip, 2008). Despite few examples of disease-induced extinction in scientific literature, disease can pose a serious threat to wildlife, driving local population extinctions through severe population declines and frequency-dependant transmission (de Castro & Bolker, 2005). Chytridiomycosis and ranaviral disease, for example, has caused mass deaths in wild populations of amphibians such as the boreal toad *Bufo boreas* (Daszak *et al.*, 1999). In a multiple-host situation, conservation efforts can be undermined if the endangered host species is likely to transmit disease to humans or livestock (Gortazar *et al.*, 2007).

There are three generally agreed options for wildlife disease management: prevention, control and eradication (Wobeser, 1994). Prevention includes all actions designed to stop the spread of disease into unaffected areas; control comprises measures to reduce the prevalence of infection, and eradication involves the total removal of an existing disease from an area. Management of infectious disease is based on the principal that if the infectious agent's reproductive rate (R_o) is less than 1, the rate of infection will be too low for the disease to persist. (Wobeser, 1994; Delahay *et al.*, 2009). Within the above options there are three main targets for management: the agent itself, the host, or the environment (Wobeser, 1994; Delahay *et al.*, 2009). These will each be discussed in detail, following consideration of the rationale for disease management in wildlife and how to choose the most effective management programme.

2. THE RATIONALE FOR DISEASE MANAGEMENT

The fundamental reasons for managing wildlife disease are always routed in an anthropocentric motive (Wobeser, 1994). The primary goal in the EU's Animal Health Strategy, for example, is 'To ensure a high level of public health and food safety by minimising the incidence of biological and chemical risks to humans' (European Commission, 2007). Other reasons to intervene in wildlife health include for the protection of domestic animal health and welfare, safeguarding trade and the wider economy, and the protection of biodiversity and threatened species (DEFRA, 2009b), all of which promote human interests.

For the smooth-running of a management programme, it is essential that the rationale for wildlife disease management is effectively communicated to those it might affect. For example, many wild birds and mammals are viewed with great affection by the general public, who can often react unfavourably when management strategies are enforced, especially since visible signs of disease are often rare (Artois *et al.*, 2001). Strong opposition to management methods from the un-informed public can make the rationale for disease control difficult to justify, especially since any lethal control methods are commonly perceived by the general public as inhumane, whilst non-lethal methods are considered humane (Reiter, Brunson & Schmidt, 1999). In addition, media reporting of such management can focus more attention on animal welfare than is helpful to the task of management (Artios *et al.*, 2001).

Since disease is a natural component of healthy ecosystems, the reasons necessitating wildlife disease management should be clarified. Unfortunately, every wild animal now lives in an environment modified to some extent by man (Wobeser, 1994). Much of these environmental changes are regrettably detrimental to the dynamic balance in which nature exists, affecting both the emergence of new diseases and their course (Daszak *et al.*, 2001; Williams *et al.*, 2002). For example, the already declining northern leopard frog *Rana pipiens* has been found to have increased exposure and susceptibility to tremadode infection due to the use of atrazine, a common agrochemical (Rohr *et al.*, 2008). Global climate change, too, is likely to cause major changes to the geographic ranges of some diseases (Daszak *et al.*, 2000), with the spread of pathogens aided by international air travel, global trade and environmental deterioration (Delahay *et al.*, 2009). Thus, it seems that disease management in wildlife is, as Wobeser (1994) phrases it, more a necessary 'exercise in mitigation' to reduce the grievous impacts of human activity, rather than an interference to the balance of nature.

3. CHOOSING THE BEST MANAGEMENT PROGRAMME

a) *Feasibility*
Once a clear rationale for the management of a disease in wildlife is established, there are several other factors to consider before selecting and embarking on a programme; firstly, whether management is actually feasible. Methods of disease control in wildlife differ considerably to those which can be used in domestic animals (Artois *et al.*, 2001). Indeed, since wild animals are free-living, management options may be severely limited by an inability to properly identify, access, or control individuals. For example, whilst it is relatively easy to restrict the movements of domestic poultry to reduce the risk of spreading HP avian influenza, controlling the movement of waterfowl and other free-ranging birds is not feasible (Gortazar *et al.*, 2007), so other options must be considered. Likewise, individual-based eradication programmes are of little or no use (Lanfranchi *et al.*, 2003).

Because of these issues, it is imperative that wildlife disease management is conducted at the population level, whilst considering the disease in context of the ecological community as a whole (White & Lowe, 2008; Lanfranchi *et al.*, 2003). This is becoming increasingly recognised through the formulation of multidisciplinary research teams and international meetings (Artois *et al.*, 2001; Gortazar *et al.*, 2007). For example, in 1998 the European Commission sponsored a meeting of experts to discuss the relative merits of two options for the control of classical swine fever in wild boar. Whilst it was important to evaluate the effectiveness of the two methods (depopulation via hunting and vaccination), it was emphasised that any control measures should be carried out on the entire meta-population of wild boar, with profound consideration of the structure and dynamics of the wild boar population in their local habitat, thus taking into account all the ecological relationships (Laddomada, 2000). Different types of disease will require different strategies, and perhaps different methods at various stages in the management programme (Wobeser, 2002). Choosing between different management options can be greatly aided by assessing the risks involved and conducting a cost:benefit analysis to

provide an evaluation of the feasibility and merit of each option (Wobeser, 1994). A cost:benefit analysis should take into account the positive and negative effects of all the ecological implications of different management strategies, and the economic feasibility. In most cases there are of course many other positive and negative factors associated with the presence of wildlife populations aside from the negative effect of disease (reviewed by Macmillan & Phillip, 2008). In addition, it is important to regard any policy or legislation that might restrict certain management options. For example, the European badger *Meles meles* is currently a protected species, outlawing lethal control as a method of management.

b) *Assessing disease distribution and ecology*
An effective monitoring and surveillance programme is essential to assess the distribution of a disease prior to initiating a management programme and provide accurate information on which to base decisions (Artois *et al.*, 2001; Gortazar *et al.*, 2007; Delahay *et al.*, 2009). For example, the prevention of outbreaks of tularaemia (a zoonotic disease maintained in wildlife populations) relies on effective surveillance and monitoring alongside hygienic measures, because complete eradication seems unachievable (Vaissaire *et al.*, 2005; Gortazar *et al.*, 2007). Since wildlife are not in any particular ownership, wildlife health monitoring is generally limited and inconsistent; some regions with none at all (Lanfranchi *et al.*, 2003; Gortazar *et al.*, 2007). International organisations, such as Office International des Epizooties (OIE) and the World Health Organisation (WHO) provide information about the world's animal health situation, but although recommended by the European Union, few countries in Europe have organised wildlife disease surveillance strategies (Artois *et al.*, 2001). In addition, Jones *et al.* (2008) report poor allocation of global resources to identify new diseases, with the majority of surveillance efforts focused on areas where diseases are least likely to originate. Hence there is an obvious urgent need for a more cohesive surveillance and monitoring system.

Predictive approaches can be extremely useful to aid understanding of the factors affecting disease ecology, especially when inadequate surveillance and monitoring leaves significant knowledge gaps (Gortazar *et al.*, 2007; Jones *et al.*, 2008). Ideally, mathematical modelling should be used in combination with surveillance techniques, rather than in isolation. Caley and Hone (2005), demonstrate the value of this application for assessing *Mycobacterium bovis* infection in feral ferrets *Mustela furo* in New Zeland. Combining modelling of the relationship between ferret population density and the force of *M. bovis* infection with observational studies of ferret scavenging behaviour, allowed estimation of the basic reproductive rate of *M. bovis* in ferret populations, and ultimately more effective management. Predictive modelling is equally valuable to test and compare the efficiency of different management methods (Barlow, 1996; Smith & Cheeseman, 2002), and to study the economics, key parameters of transmission, and conservation implications associated with a particular disease, such as with rabies (Sterner & Smith, 2006). Modelling can also help to predict where emerging infectious diseases are most likely to originate, and so promote better allocation of global resources for wildlife disease prevention (Jones *et al.*, 2008).

c) *Other considerations*

Aside from accessing adequate accurate information about the feasibility and ecological implications of management options, the final decision to adopt a particular strategy (and the future success of the programme) is significantly dependant on the interests of those involved and the support of stakeholders (Wobeser, 1994; Reiter *et al.*, 1999; Gortazar *et al.*, 2007). It is therefore important to invest in informing and convincing those concerned, and to have a sound scientific basis behind any options suggested which might create or increase conflicts between different stakeholders (Gortazar *et al.*, 2007). Whatever the strategy, there must be a clear start and end point of management, and effective methods to assess progress. It is important to note that in some cases the option of not attempting active management may be entirely appropriate, when other options have been assessed to be of less merit (Wobeser, 2002).

4. TARGETING THE INFECTIOUS OR PARASITIC AGENT

Management aimed at the infectious or parasitic agent of disease endeavours to prevent the agent from coming into direct or indirect contact with susceptible host individuals, or to reduce the effect of an agent already established in a host (Wobeser, 1994; Delahay, *et al.*, 2009). This can be done by reducing the number of susceptible individuals via immunisation, or by attacking the agent itself, either outside of its host or via medication to the host.

a) *Immunisation*

Prophylactic vaccination has majorly contributed to the successful control of many diseases in humans, and, in the last 15 years has transpired as a valuable option for disease management in wildlife also (Cross, Buddle & Aldwell, 2007). Herd immunity can be achieved when a significant proportion of the population is immune for a significant amount of time, reducing disease transmission to an unsustainable threshold at which the disease cannot persist (Wobeser, 2002; Delahay *et al.*, 2009). Consequently, vaccination is more appropriate for relatively long-lived host species, and against infectious microparasites (where host immunity is generally long-lasting), rather than macroparasites (where host immunity is short-lived) (Wobeser, 2002). Requiring a huge investment to develop, vaccination is only considered for use in wildlife when the disease in question poses a major threat to public health, the economy or endangered species, and other options are less feasible (Cross *et al.*, 2007). The management of rabies in Europe, Canada and North America provides the greatest testament to the success of wildlife vaccination to date. Rabies is a fatal disease of mammals which presents a widespread threat to global human health (Sterner & Smith, 2006). After over 20 years of extensive research (and subsequently field trials) in both Europe and North America, a major reduction in rabies incidence was detected by the mid 1990s due to oral vaccination of target host species (Brochier *et al.*, 1996). This was particularly successful in eradicating the disease from Northern Europe, where oral vaccination could be focussed on a single host species, the red fox *Vulpes vulpes*. In North America complications due to the abundance of multiple host species have so far prevented complete control of the disease (Cross *et al.*, 2007). Research is currently focussed on targeting variants of

the rabies virus, found for example in skunks, for which the current vaccine is ineffective (Weyer, Rupprecht & Nel, 2009). Following this progress, vaccination is also being explored as an option for several other diseases, notably bovine tuberculosis (see table 1).

Vaccination has great potential for the management of endangered species, such as the Ethiopian wolf *Canis simensis* which is also threatened by repeated rabies outbreaks, since depopulation is not an option (Haydon *et al.*, 2006). A vaccination programme may also avoid detrimental host behaviour changes such as increased immigration or dispersal (Gortazar *et al.*, 2007). The European badger *Meles meles*, for example, is implicated in the persistence and increase of bovine tuberculosis (TB) in UK cattle herds, through providing a reservoir for the *M. bovis*, the infectious agent (Courtenay *et al.*, 2006; Hope & Villarreal-Ramos 2008). As a result, depopulation via culling formed a significant part of the TB control policy from the 1970s until the Randomised Badger Culling Trial (RBCT) was instigated in 1998 to assess its effectiveness (Donnelly *et al.*, 2006). The results of the RBCT and subsequent new analyses suggest it is likely that culling provoked increased immigration into new areas, disruption of territoriality and increased mixing of social groups of badgers, which could explain its failure to consistently reduce cattle TB breakdown incidence (Carter *et al.*, 2007; Donnelly *et al.*, 2006). Vaccinating badgers against TB would avoid such disruption of their social structure, and after significant progress made in the last 10 years, is now a realistic goal. The BCG vaccine, which is currently being developed for cattle and other wildlife host species too, is expected to be licensed for use in badgers this year, and the first stages in the vaccine deployment project have already begun (Hope & Villarreal-Ramos 2008; DEFRA, 2009c).

An effective vaccine requires an effective immunogen and deployment method. Current delivery methods include remotely by injection, and orally via baits (Cross *et al.*, 2007). Both can present a logistical challenge for use in free-living wildlife. Oral bait is presently the most successful method, if it can be species specific (Artois *et al.*, 2001). The development of oral vaccines against both classical swine fever and

Table 1 Wildlife vaccines available

Disease	Targeted host(s)	Risk justifying vaccine deployment
Rabies	Red fox, racoon dog, arctic fox, skunk	human health risk
Lyme disease	White-footed mice	human health risk
Plague	Black-tailed prairie dog	human health risk
Bovine tuberculosis	Eurasian badger	economic risk to livestock production
Classical swine fever	Wild boar	economic risk to livestock production
Brucellosis	Elk, bison	economic risk to livestock production
Aujeszky's disease	Feral pigs	economic risk to livestock production
Myxomatosis	Rabbit	risk to threatened species
Pasteurellosis	Big horn sheep	risk to threatened species
Anthrax	Cheetah, black rhino	risk to threatened species

(Based on Cross *et al.*, 2007 and Gortazar *et al.*, 2007)

brucella for feral swine, have been hindered by problems with vaccine safety for non-target species which might inadvertently consume baits (Artois *et al.*, 2001; Davis & Elzer, 2002). Since immunisation is often ineffective against those already infected, susceptible individuals must be vaccinated before the average age of infection in the population (Wobeser, 2002). This can present a challenge for some diseases which are transmitted vertically from parents to offspring, or for host species such as wild boar, where adults take baits more preferentially than young (Laddomada, 2000). The evolutionary implications of vaccination also need addressing. Concerns have been raised over the potential for adverse evolutionary consequences, such as inhibiting selection of disease resistant characteristics on the host (Delahay *et al.*, 2009). In addition, vaccination may cause an increase in host density (due to improved health), providing more opportunity for other diseases to attack, and lowering the threshold at which such disease can persist (Smith & Cheeseman, 2002).

b) *Attacking the agent itself*
For diseases caused by non-infectious agents, such as toxic substances, management can directly focus on agent elimination. In this case, measures can be taken to reduce the prevalence of harmful substances in the environment, normally involving changes in human behaviour (Wobeser, 1994). For example, avoiding wildlife disease outbreaks as a result of pollution of the aquatic environment by waste human and veterinary pharmaceuticals highlights the importance of effective sewage treatment (Morley, 2009). Attacking an infectious agent is best done through medication to the host. Since this is expensive, greatly limited by practicalities, and not always successful, treatment of sick wild animals can only be justified in exceptional circumstances, such as for severely endangered species. For example, when a major oil leak contaminated over 48 km of Puerto Rico's shore in 1994, thousands of dead and alive oiled organisms (including cnidarians, annelids, crustaceans, molluscs, echinoderms, fishes, birds and sea turtles) were washed ashore. Those alive were cleaned and treated, including four types of endangered or threatened species. Despite these efforts, only 63% were successfully rehabilitated (Mignucci-Giannoni, 1999). The medication of wild animals creates similar concern as vaccination, with analogous potential for undesirable evolutionary consequences and unnaturally elevated host densities. Poor medication could also encourage resistance to build up, and harmful drugs to persist in the environment (Delahay *et al.*, 2009).

Both vaccination and medication present a more ethically attractive option for the control of wildlife disease than other forms of host depopulation management. Whilst it seems wildlife medication is unlikely to become a feasible option for wide use in free-ranging populations, the success of vaccination so far, and the current research effort give it great potential to be an increasingly important management tool in future.

5. TARGETING THE HOST

Historically, the most used wildlife disease management strategies target host species. For a non-infectious disease, management attempts to prevent the host species accessing the cause of the disease, by manipulating its distribution. For an

infectious disease, this form of management aims essentially to reduce host density to an extent at which the frequency of disease transmission is too low for the disease to persist. This can be also be done by manipulating host distribution, in addition to culling, or reducing reproduction (Delahay *et al.*, 2009).

a) *Manipulating distribution*
Manipulating distribution involves the dispersion of animals from the area of disease outbreak. This could be useful to control the spread of disease in newly colonising populations, but is easiest accomplished for localised outbreaks of non-infectious diseases, since to avoid increasing the transmission of infectious diseases, only confirmed uninfected individuals could be dispersed (Bar-David *et al.*, 2006; Delahay *et al.*, 2009). There are very few such examples in the literature, and even aside from the intensive labour required, in practice, this method provides limited value for the effective control of wildlife disease, since repopulation of the dispersed area is common (Wobeser, 1994).

b) *Culling*
Widespread depopulation can eradicate a disease from a defined area if the number of infectious individuals is reduced to below a threshold at which the disease cannot persist (Delahay *et al.*, 2009). This method can be successful in isolated populations, where dispersal is limited. Foot-and-mouth disease was successfully eradicated from a Californian national park, for example, following a deer culling programme (Ward, Laffan & Highfiield, 2007). However, there are few examples where culling on a larger scale has successfully reduced disease incidence, and increasing evidence of rapid re-establishment of high population densities due to compensatory reproduction and immigration (Artois *et al.*, 2001). This has been confirmed for example in wild boar following lethal control to combat classical swine fever (Laddomada, 2000). Whilst selective culling can be an effective tool for disease control in livestock (Sutmoller *et al.*, 2003), its use in wildlife is limited since infected individuals are often difficult to distinguish (Wobeser, 2002). Consequently, culling is becoming increasingly perceived by disease ecologists as a less effective disease management strategy (Gortazar *et al.*, 2007). Methods of culling (including hunting, trapping, gassing and poisoning) can be labour intensive and costly for large scale disease control, and can have implications for animal welfare (Delahay *et al.*, 2009). In addition, the wider ecological consequences of culling are uncertain and need further investigation (Cross *et al.*, 2007), and public controversy over such management, as in the example of culling badgers for the control of bovine TB, can also affect success (Enticott, 2001).

c) *Reducing reproduction*
Controlling fertility has great potential for reducing host population density and consequently disease transmission (Killian *et al.*, 2007). Whilst its application to wildlife disease management is relatively new, various methods have been tested in almost 50 animal species, some of which could be easily made available for wildlife (Barfield *et al.*, 2006). Reducing mating and parent-offspring contact can significantly decrease disease transmission opportunities, and place less reproductive burden on mothers, potentially improving their physical condition and resilience to disease

(Delahay *et al.*, 2009). Depopulation by this method would be less immediate than culling, but may be less disruptive to social structure, allowing natural density dependent mechanisms to continue to regulate population dynamics. This might avoid the rapid repopulation that sometimes follows after lethal control (Killian *et al.*, 2007; Delahay *et al.*, 2009). Contraception of animals is a highly invasive process which should be taken with caution. Currently immunocontraceptives have the greatest potential for success. Other techniques, such as chemical or hormone agents, surgical sterilisation or interuterine devices, are less feasible for use in wild populations because of the heavy practical or financial requirements (Delahay *et al.*, 2009). Fertility control is likely to be most beneficial in combination with other methods, such as culling to more rapidly reduce initial host density, or vaccination to simultaneously target the disease agent too (Smith & Cheeseman, 2002; Barfield *et al.*, 2006). Indeed, recent developments show promising potential for incorporating immunocontraception into rabies vaccines, with little extra cost (Wu *et al.*, 2009). Other research has revealed potential for fertility control to reduce the incidence of diseases such as bovine TB in brushtail possoms (Caley & Ramsey, 2001) and white-tailed deer (Malcolm *et al.*, 2010), although further research is needed. The public view of fertility control is generally more favourable than for culling (Killian *et al.*, 2007); its use could therefore help cultivate better public support for wildlife management.

6. TARGETING THE ENVIRONMENT AND INFLUENCING HUMAN ACTIVITIES

The environment can be defined as the conditions in which an organism lives, including the influences of biotic and abiotic components (Delahay *et al.*, 2009). Wildlife populations and disease dynamics can be greatly influenced by environmental change (Williams *et al.*, 2002). Since such change is principally anthropogenic (Wobeser, 1994), and making desired alterations to the environment generally involves a change in human behaviour, management methods targeting the environment are considered here together with influencing human behaviour. Effectively managing wildlife disease through the environment requires knowledge of the impact of such management on disease dynamics. This is, as yet, little studied, forcing decisions to be based on less-definite correlative data (Delahay *et al.*, 2009). Because environmental factors (such as geographical barriers, habitat corridors and migratory routes) can also influence the success of alternative wildlife disease management strategies, the need for this research is paramount. Methods of environmental management to reduce disease incidence generally fall into three categories: manipulating habitat carrying capacity to reduce or increase host density; habitat modification with a view to reduce host contact with pathogens or control disease vectors; and those associated with conservation.

a) *Manipulating environment carrying capacity*
Reducing the carrying capacity of an environment is an alternative method of reducing host density (to those that target hosts directly). In fact, as Wobeser (2002) states, direct host depopulation without reducing the habitat carrying capacity is only a temporary measure. Reducing habitat carrying capacity can be achieved by limiting the availability of critical resources such as food or shelter, for example via feeding bans, which have been successful in preventing ungulate aggregation, and

potentially disease transmission (Gortazar *et al.*, 2007). However, this technique is not without its drawbacks. Firstly, host population reduction by this method takes longer to achieve compared to alternatives acting directly on the host, such as culling (Wobeser, 2002). Any population reduction would also be preceded by a period of host malnutrition, increasing disease susceptibility and potentially raising issues of animal welfare. In addition, individuals would be more likely to disperse to find other resources, increasing the risk of disease transmission (Delahay *et al.*, 2009). Consequently, in some situations it may actually be of greater benefit to increase habitat carrying capacity, to increase host health and resilience to disease (Gortazar *et al.*, 2007). Nevertheless, any modification of critical resources must only be done with careful consideration of the disease dynamics and wider ecosystem impacts (Delahay *et al.*, 2009).

b) *Habitat modification*
Geographical features, including barriers such as rivers, roads and lakes, can influence the spatial distribution of disease in wildlife (Van Buskirk & Ostfeld, 1998). Manipulating the landscape within the desired area for disease control could therefore slow the spread of disease. This can be indirectly targeted at host species to reduce intra- and inter-specific contact or avoid contact with a disease agent. Implementing farm biosecurity measures, such as using electric fencing to exclude badgers or deer from buildings, has also been effective, potentially decreasing TB transmission to livestock with minimal disruption to wildlife social territories (Kaneen *et al.*, 2002; Tolhurst *et al.*, 2008). Such environmental manipulation targeting hosts may be restricted to small scale changes (such as biosecurity strategies tailored to individual farms) because many wild animals can make complex decisions about movement and distribution, so behavioural outcomes are difficult to predict (Delahay *et al.*, 2009). Environmental modification can also be targeted at disease vectors by depleting their critical habitat. For example, drainage of stagnant water decreases the larval habitat for malaria carrying mosquitoes (Kilama, 2009). Unfortunately, this approach can have limited effect if a disease has multiple vectors (Gortazar *et al.*, 2007). For both host and vector targeted environmental management, financial feasibility and accidental impacts on non-target species must also be considered.

c) *Conservation related environmental management*
Substantial environmental change caused by humans, such as habitat fragmentation, degradation, or spatial heterogeneity, can disrupt the equilibrium of a naturally occurring host-parasite system and provoke a disease outbreak (Augustine, 1998; Williams *et al.*, 2002). Habitat fragmentation resulting from expanding agriculture, urbanisation and deforestation is a particularly significant threat. The subsequent reduction in wildlife habitat can modify host species distributions including contact rates with other wild species, humans and livestock (Delahay *et al.*, 2009). For the severely endangered Ethiopian wolf *Canis simensis*, habitat fragmentation combined with rabies and canine distemper outbreaks greatly increases extinction risk (Haydon, Laurenson & Sillero-Zubiri, 2002). Habitat degradation by pollution or physical alteration can also directly cause disease or increase wild animals' disease susceptibility. For example, DDT persistence in the environment for over 40 years

continues to threaten the health of fish and piscivorous birds (Hinck *et al.*, 2009). Spatial heterogeneity can encourage the persistence of diseases, such as Lyme disease. Whether a tic vector is carrying the disease varies with complex factors associated with local habitat features (Van Buskirk & Ostfeld, 1998). In light of these issues it is unsurprising that most conservation efforts to improve habitat quality or species richness can also have a positive effect on reducing disease emergence (Jones *et al.*, 2008), and should be supported. Again, any changes to the environment must be done with caution; not all favoured methods for conservation will necessarily be beneficial for disease control. The creation of habitat corridors, for example, may promote the persistence and spread of disease (Delahay *et al.*, 2009).

d) *Influencing human activity*
Most methods of disease management through targeting the environment are highly dependant on human activity, policy or public perception. This highlights the importance of having the support of stakeholders, achieved through campaigns and education to help resolve human-wildlife conflict (Gortazar *et al.*, 2007). For this to be successful it is essential to understand stakeholder attitudes and influences behind their decision making (Delahay *et al.*, 2009). In addition to targeting the environment, disease agents, or hosts, simple changes in human behaviour such as the removal of diseased carcasses could significantly decrease disease transmission in wildlife (Delahay *et al.*, 2009). For example, the introduction of basic cleaning and disinfecting habits helped to control avian influenza in Europe (Yee, Carpenter & Cardona, 2009). Unfortunately management decisions are not always made by those best informed, or can be coloured by other political agendas (Gortazar *et al.*, 2007). This can make for ineffective management, as demonstrated in the early attempts to control wildlife brucellosis in Wood Buffalo National Park, Canada. To reduce plains bison *B. bison bison* population density without provoking the public outcry seen previously with culling, part of the brucellosis-carrying herd was translocated to a brucellosis free area, despite opposition from scientists. Unsurprisingly the disease spread and continues to persist (Peterson, 1991).

7. CONCLUSIONS AND FUTURE DIRECTIONS

Management of wildlife disease must be based on sound scientific knowledge; most crucially knowledge of disease dynamics and the relationships with hosts, vectors and the wider ecosystem. Since current knowledge is far from adequate, continued research into this is essential, and surveillance schemes should be extended to more comprehensively cover species and areas that have so far been neglected. There is also still much to learn about the effects of different management techniques, highlighting the importance of proper monitoring and assessment of current methods to better inform future management decisions.

There is no blanket strategy approach to wildlife disease management. Techniques may include host vaccination, medication and depopulation via dispersal, culling or fertility control, and environmental manipulation. Determining the suitability of different strategies requires effective analysis of feasibility, costs, and benefits, and predictive modelling should be utilized more widely to actually inform management.

It is also important to take into account the views of stakeholders, combining management with educational campaigns, to avoid conflicts.

There is great need for an organised framework for the basic co-ordination of the management process. The formulation of multidisciplinary research teams will aid this co-ordination and promote more effective communication of information between geographical areas and disciplines, ultimately to give policy makers a more comprehensive understanding of the management issue to make the best decisions.

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Section 2: Research Project

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The implications of bite wounding for disease status in badgers *Meles meles*

Abstract

Aggressive interactions between individuals can pose a significant risk for disease transmission. Positive associations between bite wounding and disease status in the Eurasian badger *Meles meles* have led to the suggestion of bite wounding as an alternative route of *Mycobacterium bovis* (bovine TB) infection to aerosol infection of the respiratory system. Understanding the social behaviours behind transmission routes is important for the effective management of infection. This study investigated the incidence of bite wounding in a naturally infected badger population with regard to (1) the evidence for bite wounding as a route of bovine TB transmission between badgers, and (2) the social behaviour of infected and uninfected animals. The results were consistent with bite wounding being a true route of transmission: *M. bovis* infected bite wounds were randomly distributed across all body locations, independently of those locations normally associated with respiratory infection, and positively associated with the incident event of bovine TB infection in badgers, with no association to positive respiratory-related cultures. Badgers with established disease (as detected by microbiological culture) were more likely to have bite wounds than those that were culture negative, which may be a reflection of abnormal increased ranging behaviour of infected badgers. Both bite wound infected individuals and infected individuals with bites may be important for bovine TB transmission to cattle and should be taken into consideration when planning a bovine TB management programme.

Key words: Bite wounding, Eurasian badger, wildlife disease, bovine tuberculosis, *Mycobacterium bovis*, transmission, social behaviour.

Introduction

Managing wildlife disease is a huge task growing in importance and recognition globally (Artois *et al.* 2001). The current and potential future impacts of wildlife disease on global health, agriculture and biodiversity are becoming increasingly more widely recognised (Daszak, Cunningham & Hyatt 2000; Wobeser 2002; Delahay, Smith & Hutchings 2009). A clear understanding of the routes of disease transmission in wildlife and the behavioural factors that affect them are essential for developing the most effective management technique.

Aggressive interactions between individuals can pose a significant risk for disease transmission, particularly within territorial species. In carnivores, several viral diseases are known to be transmitted by direct contact involving aggression between individuals (see Murray *et al.* 1999), such as rabies in red foxes (Krebs, Wilson & Childs 1995) and feline immunodeficiency in feral cats (Courchamp *et al.* 1998). Aggressive interactions leading to bite wounding among Eurasian badgers *Meles meles* has also been implicated as a transmission route for the bacterial infection bovine tuberculosis (TB), the management of which is particularly economically costly in the UK.

Badgers are a natural reservoir of *Mycobacterium bovis*, the causative agent of TB, and infection is endemic in badger populations across southwest Britain (Cheeseman, Wilesmith & Stuart 1989). Microbiological culture of faeces, urine, sputum, pus from discharging lymphnodes and bite wound swabs from infected individuals has intermittently detected *M. bovis* (Clifton-Hadley, Wilesmith & Stuart 1993), indicating the potential for some individuals to excrete the bacteria via multiple routes. Consequentially, badgers are implicated in the persistence of TB in UK cattle herds, a serious problem particularly in southwest Britain (Krebs *et al.* 1997). Despite badger culling forming a component of TB control policy between 1973 and 1998, TB in cattle has continued to increase (Donnely *et al.* 2007; Delahay *et al.* 2009b). Understanding social behaviour in badger populations and all possible routes of *M. bovis* transmission between badgers is therefore critical to the development of sustainable disease management plans.

The study of bite wounding in badgers can give insights into badger behaviour, and further the understanding of the transmission of TB. It is widely accepted that the primary route of *M. bovis* transmission among badgers is by aerosol infection of the respiratory system (Clifton-Hadley *et al.* 1993). However, studies by Gallagher & Nelson (1979), Clifton-Hadley *et al.* (1993), and Jenkins *et al.* (2008) have documented positive associations between the presence of bite wounds and *M. bovis* infection, and suggest that bite wounding may be an alternative infection route. Badgers with severe pulmonary TB may have contaminated saliva, and a bite from such an individual could inoculate the recipient with *M. bovis* bacilli (Gallagher & Clifton-Hadley 2000).

Bite wounds can occur among badgers as a result of aggressive territorial and reproductive behaviour (Cresswell *et al.* 1992; Macdonald *et al.* 2004). Badgers commonly live in territorial social groups in parts of the UK where densities are

relatively high (Kruuk 1978). Whilst it has been suggested that social group boundaries may be maintained passively via latrine marking (Stewart, Anderson & Macdonald, 1997), fighting both within and between groups is a regular feature of badger society, particularly at high densities (Macdonald *et al.* 2004). Aggressive encounters within social groups are thought to be related to the protection of young, competition for breeding status (Cresswell *et al.* 1992; Gallagher & Clifton-Hadley 2000), or social tension (Macdonald *et al.* 2004).

Bites to the head, neck, body, limbs and rump have commonly been recorded in free-living badgers (e.g. Gallagher & Nelson 1979; Macdonald *et al.* 2004; Delahay *et al.* 2006), with those on the rump and body most likely to occur during fighting which has escalated beyond the initial head-to-head exchanges, sometimes leading to severe wounding (Gallagher & Nelson 1979; Macdonald *et al.* 2004). Adult male badgers may be more likely to reach this stage of aggression, indicated by the majority of male bite wounds occurring on the rump (Macdonald *et al.* 2004; Delehay *et al.* 2006). In contrast, females are more likely to be bitten on the head (Delehay *et al.* 2006), which is thought to be due to male aggression during mating, and their defence of cubs within the sett (Neal & Cheeseman 1996, cited in Delahay *et al.* 2006).

To positively identify bite wounding as a true route of TB transmission, direct experimental evidence is required. However, difficulties in detecting disease and the complex nature of TB expression make such evidence difficult to obtain. An alternative explanation is that bite wounds could represent a secondary site of infection for *M. bovis* which has been previously established by a different route, such as respiratory. Bacilli may then preferentially congregate at wound sites as part of the normal haematogenous spread of disease (Clifton-Hadley *et al.* 1993). However, *post mortem* examination of badgers suggests that the pattern of disease progression to different body locations following TB infection is different depending on the route of infection (Corner, Murphy & Gormley 2011). Several different studies describe gross pathological evidence of a distinctive kind of TB infection associated with the presence of bite wounds (Gallagher & Nelson 1979; Clifton-Hadley *et al.*

Figure 1. Fresh bite wounds located (a) on the rump, and (b) the jaw of a badger undergoing routine sampling at Woodchester Park, Gloucsetershire.

1993; Jenkins *et al.* 2008). The resulting disease appears to be particularly severe and acute (Gallagher & Nelson 1979; Gallagher & Clifton-Hadley 2000), and is associated with shorter life-expectancy (Clifton-Hadley *et al.* 1993). This, coupled with the common discovery of tuberculous abscesses at wound sites could indicate the haematogenous spread of disease directly from infected bite areas (Gallagher & Nelson 1979; Clifton-Hadley *et al.* 1993), as seen in the experimental intradermal inoculation of captive badgers (Pritchard, Stuart & Brewer 1987).

The discovery of TB infected bite wounds in areas of the body where respiratory originated infection does not normally spread could therefore support the notion of bite wounding as a transmission route. For example, the solitary detection of *M. bovis* in peripheral lymph nodes, such as the prescapular or popliteal lymph nodes, has previously been attributed to infection via bite wounding (Fagan 1993, cited in Gallagher & Clifton-Hadley 2000; O'Boyle 1998, cited in Gallagher & Clifton-Hadley 2000). Infection resulting from aerosol transmission is most commonly associated with the lung and pulmonary lymph nodes. Similarly, detection of *M. bovis* in the head lymph nodes has been linked to infection of the upper respiratory tract (Gallagher & Clifton-Hadley 2000; Murphy *et al.* 2010), although in advanced disease further dissemination of respiratory infection to other parts of the body can occur via the lymphatic system or blood (Gallagher & Clifton-Hadley 2000). If bite wounding is a true route of infection, and there is no bias in the locations of bites given by TB infected individuals, the isolation of *M. bovis* would be expected from bite wounds in all body locations, not just those normally associated with respiratory infection. Furthermore, the isolation of *M. bovis* from bite wounds in the absence of any culture evidence of respiratory infection (e.g. from the culture of sputum or faeces samples), or in areas of the body that are not closely associated with lymph nodes, such as the rump, would be consistent with bite wound transmission.

Knowledge of how infected animals behave is central to understanding how TB is transmitted between individuals (by bite wounding or other routes), maintained within badger populations, and passed on to other species. Behavioural factors such as social interaction, movement within and between social groups, and establishing and defending a territory can have a profound effect on disease dynamics (Rogers *et al.* 1998; Vicente *et al.* 2007). Badgers can cope seemingly well with chronic infection, surviving for long periods of time and producing cubs (Cheeseman, Wilesmith & Stuart 1989; Clifton-Hadley 1993). However, at some point a badger's ability to cope with infection appears to rapidly decrease, followed by a huge multiplication of *M. bovis* and rapid demise (Gallagher, Muirhead & Burn 1976; Pritchard *et al.* 1987). In line with this, there is evidence to suggest that the behaviour of infected badgers becomes increasingly abnormal as disease progresses. Garnett, Delahay and Roper (2005) found tuberculous badgers' home ranges extended four times greater into neighbouring territories and were 50% larger than those of TB negative individuals. In addition diseased badgers have been found to range over a greater proportion of their territory and forage further away from their home sett (Cheeseman & Mallinson 1981; Garnett, Delahay & Roper 2005). Since movement between social groups can increase the likelihood of aggressive territorial encounters (Kruuk 1978), there may be heightened opportunity for diseased badgers

to transmit TB via bite wounding and aerosol routes. Indeed, there is evidence both that the probability of detecting bite wound scars is affected by a badger's movement history (Macdonald *et al.* 2008), and that increased movement between social groups can increase the incidence of infection (Rogers *et al.* 1998; Vicente *et al.* 2005). Any consistent difference in the ranging behaviour of animals with progressive disease might potentially be reflected in the amount of bite wounds they receive. The incidence of bite wounding is known to also be affected by gender, age and season (Macdonald *et al.* 2004; Delahay *et al.* 2006).

In the present study, the incidence of bite wounding in a naturally infected badger population was investigated with regard to the evidence for bite wounding as a transmission route between badgers, and the social behaviour of TB positive and negative individuals. All analyses were carried out using data on badgers captured during the long term study of the badger population in Woodchester Park. In line with the existing evidence supporting bite wounding as a route of TB transmission, it was hypothesised that:
- the proportion of TB positive bites would not differ by location on the body, but would rather be randomly distributed.
- there would be a positive association between a TB positive bite wound recorded at any point in a badger's excreting lifetime also being recorded as present (and culture positive) at the individual's incident event of TB infection.
- there would be no association between positive bite wounds and positive respiratory cultures at the TB incident event.

In addition, given the observations that diseased animals range more widely across neighbouring territories, it was hypothesised that:
- TB infected badgers would be more at risk of having a bite wound than TB negative badgers.

Materials and methods

STUDY AREA

The study was carried out using data from a naturally infected badger population in Woodchester Park, situated on the Cotswold limestone escarpment in Gloucestershire, south-west England. At the core of the 11km^2 study area is a steep-sided, heavily wooded valley, surrounded by cattle-grazed pastoral land, with patches of arable farmland, scrub and mixed woodland. The area contains 36 badger social groups (21 to 23 of which have been intensely studied, depending on the distribution of badgers each year), and main setts are fairly evenly spaced throughout the valley (Delahay *et al.* 2000).

BADGER TRAPPING AND SAMPLING PROCEDURE

The badger population in Woodchester has been the subject of a long term epidemiological study into the dynamics of tuberculosis (see Delahay *et al.* 2000). Data for the present study was collected from 1976 to 2010. Main setts were initially trapped between two and four times a year, then from 1984 social groups were

trapped and sampled four times per year, using the following procedure. Steel mesh box traps were positioned near badger 'runs' at the active setts in each territory, and baited with peanuts for four to eight days before being set for two nights (Cheeseman & Mallinson, 1979). Badgers were anaesthetized prior to examination. This was by intramuscular injection with ketamine hydrochloride at a dose rate of 20 mg/kg (Mackintosh *et al*. 1976) during 1976 to 2004, and in subsequent years by intramuscular injection of medetomidine hydrochloride (Domitor), butorphanol tartrate (Torbugesic) and ketamine hydrochloride (Vetalar), with the option of reversing the effects of medetomidine hydrochloride with atipamezole (Antisedan) (de Leeuw *et al*. 2004). On first capture badgers were permanently marked with a unique identifying tattoo in the inguinal region (Cheeseman & Harris 1982). Data, including location of capture, age (cub, yearling or adult), sex, and body condition (poor, fair, good, or very good, based on fat coverage and musculature of shoulder blades, pelvic region, ribs and vertebrae) were recorded for each animal. Any bite wounds were documented along with their age (fresh or old), size (small (puncture), medium (gash), large (gaping)), location (head, neck, body, rump, limb, inguinal), and the amount of bites in that location (one, few, lots). TB infection status was determined according to biological culture of *M. bovis* from sputum (oesophageal and/or tracheal aspirates), urine and faeces samples, swabs of bite wounds and puss from abscesses. Badgers with one or more positive culture result were diagnosed as excreting *M. bovis* and consequently considered to be in a state of relatively advanced disease, and classified as such thereafter, regardless of the culture results from any subsequent re-capture. Culture test results were deemed the best criterion for determining infection status since it only detects individuals with established disease that excrete bacilli (Pritchard *et al* 1986). Positive sputum and faecal samples were considered as indicative of respiratory disease. After a period of recovery from anaesthesia, badgers were released at their original locations. All *post mortem* records of badger carcasses found in the study area were excluded from the current data set due to the higher sensitivity of *post mortem* examinations for detecting TB infection.

STATISTICAL ANALYSIS

This study focused on four analyses. The first three analyses investigated the evidence for bite wounding as a true route of *M. bovis* infection by investigating (1) the association between bite wound body location and TB positive bites, (2) the association between TB positive bites recorded at any point in a badger's excreting lifetime and TB positive bites recorded at the incident event of TB infection, and (3) the association between TB positive bite wounds and positive respiratory cultures at the incident event. The final analysis then investigated (4) the effect of TB status on the likelihood of being bitten. Response variables represented individual trap events, and the dataset contained repeated observations of individuals since many badgers were caught more than once. Data for the first and fourth analyses were analysed using generalized linear mixed modelling (GLMM) (Schall 1991) in GenStat for Windows XP version 13.1 (VSN International Ltd., Hemel Hempsted, UK). Statistical significance of explanatory variables was assessed with Wald tests, and non-significant terms (with less than 95% confidence) were removed from models, simplifying to the minimum adequate model. Normality of standardised residuals

was assessed using standard model diagnostic checks. Data for the second and third analyses were analysed with Pearson's chi-square test with Yates correction for continuity.

The association between bite wound body location and TB positive bites

Generalized linear mixed modelling was used to investigate variation in the TB status of fresh bite wounds of captured badgers, in relation to bite wound body location. Data was therefore limited to trap events with recorded fresh bites with a known TB status (having been swabbed and microbiologically cultured). A binomial error structure was incorporated into the model design and dispersion parameters were fixed at one. The response variable was bite wound TB status, and bite wound body location was fitted as a fixed effect. Bites in similar locations (head and neck; body and inguinal) were combined due to the subjectivity of classifying such bite locations during sampling (see fig 2.). Bite size, bite frequency and badger sex were also fitted as fixed effects, and individual was included as a random effect to control for multiple bite observations from the same badger. All possible two-way interactions were considered in the maximal model.

The association between TB positive bites recorded at any point in a badger's excreting lifetime and TB positive bites recorded at the incident event of TB infection

Pearson's chi-square test, with Yates correction for contingency, was used to investigate variation in the likelihood of TB positive bites to have also been detected as positive at the individual's incident event for TB infection (i.e. the first capture when a positive culture result was obtained). The Null hypothesis was that there was no association between the recording of TB positive bites during any point in a badger's excreting lifetime and the recording of positive bites at the individual's incident event of TB infection. This analysis included all trap events of TB positive badgers with a fresh *M. bovis* infected bite wound present. Multiple observations of the same positive wounds were deleted.
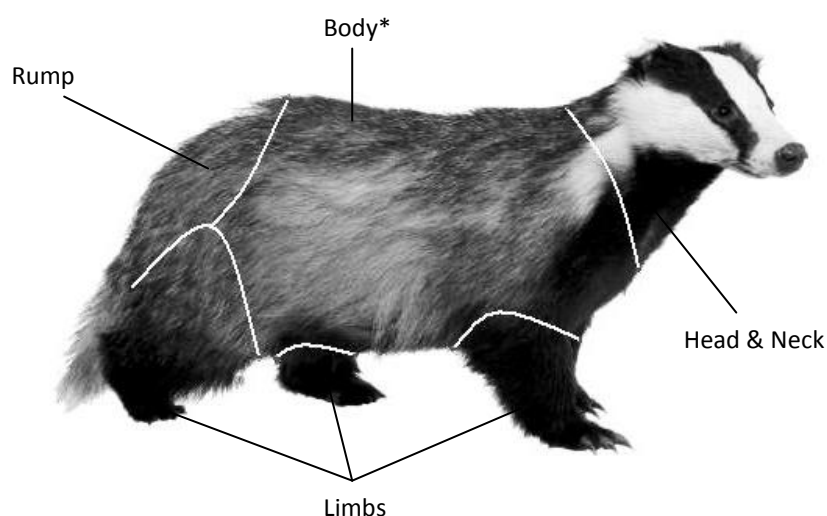

Figure 2. The location categories on a badger to which bite wounds were classified during this study.
*Body includes the inguinal region

The association between TB positive bite wounds and positive respiratory cultures at the incident event

Pearson's chi-square test, with Yates correction for contingency, was used to investigate variation in the likelihood of TB positive bite wounds to be the sole positive culture for *M. bovis* or be accompanied by positive respiratory cultures at the incident event (i.e. the first capture when a positive culture result was obtained). Respiratory cultures included positive cultures from faeces, oesophageal aspirate and tracheal aspirate samples, which were considered to be indicative of respiratory infection. The Null hypothesis was that there was no association between positive bite wounds and positive respiratory cultures at the TB incident event. This analysis included all trap events recording an incident event of TB infection where a fresh TB positive bite wound was recorded.

The association between TB infection status and the incidence of fresh bites wounds

Generalized linear mixed modelling was used to investigate variation in the likelihood of detecting fresh bites on captured badgers, in relation to their existing TB status. A binomial error structure was incorporated into the model design and dispersion parameters were fixed at one. Fresh bite incidence (0/1) was the response variable, and prior TB status was fitted as a fixed effect. Age, Sex, season of capture, number of captures, and prior body condition were also included as fixed effects, to control for variation expected to be attributed to their effects. Age (in years) was calculated from age at first capture (where cub = 0, yearling = 1 year) and year of first capture, then categorised by years into cub (<0), Juvenile (1-2), adult (3-6), and old (>6). Individuals for whom age at first capture was not known (i.e. adults at first capture) could not be included. Prior body condition (from the most recent previous capture) rather than current condition was included, since the current body condition assessment would depend, in part, on the presence of bite wounds. Observations that did not have data from a prior capture were excluded. All 15 possible two-way interactions were considered in the initial maximal model, and individual was fitted as a random effect to control for multiple observations of the same badger.

Results

Between May 1976 and September 2010 there were 13,559 trap events of 2,645 different badgers, and 280 fresh bite wound observations.

The association between bite wound body location and TB positive bites

There were 199 fresh bite wounds for which TB infection status was known, from 149 individuals. Of these, 29 bite wounds (14.6%) were TB infected. Both TB positive and negative bites were observed in all locations (head and neck, body, rump, and limb). Bite location had no effect on bite TB status (table 1). There was also no significant effect of bite frequency, bite size or badger sex on bite TB status (Table 1), and no significant interactions between factors. There were repeated observations of 33 individuals, with the most observations from one badger totalling five and occurring just once.

Table 1. The effect of bite location and other factors on the TB status of fresh bite wounds.

Variable		Number of observations	Mean percentage TB positive bite wounds	Effects	Wald, χ^2 (df)	P
Bite location	Head & Neck	79	13.8	0.00	0.85 (3)	0.837
	Body*	20	11.1	-0.85		
	Rump	82	17.1	-0.32		
	Limb	20	10.0	-0.36		
Number of bites	One	153	16.3	0.00	0.66 (2)	0.717
	Few (2-3)	35	11.4	-0.56		
	Lots (>3)	11	0.0	-19.59		
Bite size	Large	64	21.9	0.00	2.69 (2)	0.260
	Medium	76	10.5	-1.15		
	Small	59	11.9	-0.79		
Sex	Female	83	14.5	0.00	0.07 (1)	0.797
	Male	116	14.7	-0.13		

The number of observations and percentage bite wound values are from the raw data. The effects, Wald statistic and P values are generated from generalized linear mixed models, where individual badger was included as a random effect (estimate ± SE = 1.8 ± 0.8). No terms had any significant effect. *Body includes bite wounds located in the inguinal region (n=3).

The association between TB positive bites recorded at any point in a badger's excreting lifetime and TB positive bites recorded at the incident event of TB infection

There was a significant positive association between TB positive bites recorded at any point in a badger's excreting lifetime and TB positive bites at the incident event (χ^2_1 =10.7, P < 0.01), thus the null hypothesis (that there would be no association between the recording of TB positive bites during any point in a badger's excreting lifetime and the recording of positive bites at the individual's incident event of TB infection) can be rejected. Of all the capture events where a fresh culture positive bite wound was present, 83.3% were also detected at the incident event of TB detection by culture. Only 16.7% of positive bites were not present at the first culture event (Fig. 3).

The association between TB positive bite wounds and positive respiratory cultures at the incident event

There was no significant association between TB positive bite wounds and positive respiratory cultures at the incident event (χ^2_1 =2.63, P = 0.105), hence the null hypothesis (that there would be no significant association between TB positive bite wounds and positive respiratory cultures at the incident event) can be accepted. Thirteen of the 19 positive bite wounds that were present at the incident event (68.4%) represented the sole culture positive sample obtained at that point (i.e. there was no association with any other cultures to indicate respiratory infection).
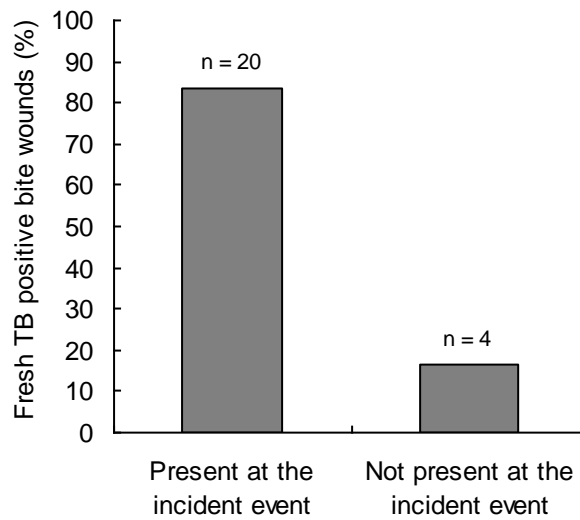
Figure 3. The percentage of TB positive bite wounds within the dataset that were and were not also present at the individual's incident event for TB infection. The incident cultures that did not include a positive bite wound culture had one or more positive culture from oesophageal aspirate, tracheal aspirate or faecal samples, indicative of respiratory infection.

The association between TB infection status and the incidence of fresh bites wounds

This analysis included 9,988 badger trap events (after discarding those of unknown age, or without data from a previous capture; see methods) of 1,717 different badgers. Of these, 123 individuals were or became infected with TB at some point during the study period. Within the dataset, 209 fresh bite wound observations were recorded. Existing TB status had a significant effect on the incidence of fresh bite wounds recorded (χ^2_1 =16.2, *P* < 0.001). Badgers with a history of *M. bovis* excretion were significantly more likely to be detected with a bite wound than those that had not previously been detected as culture positive (table 2, fig. 4a). Within the dataset, the percentage of bite wound incidents (i.e. fresh new bites) was three times higher for badgers detected as culture positive compared to those that were not. The incidence of fresh bites also varied significantly with age, with the lowest incidence of bites in cubs (<1 year olds) and the highest incidence in adults (three to six year olds) (fig. 4b); season, with least bites detected in summer, and most bites detected in winter (fig. 4c); and number of captures (table 2). Sex and prior body condition had no effect on the incidence of fresh bites (table 2). All interactions between factors were tested, and none were found to be significant, leaving the minimum adequate model as including existing TB status, age, season and number of captures.

Table 2. Variation in the incidence of fresh bite wounds in badger captures in relation to TB infection status and other factors.

Variable		Number of observations	Mean percentage bite wound incidence	Effects	Wald, χ^2 (df)	P
TB status*	Not infected	9524	1.9	0.00	7.1 (1)	0.008
	Infected	464	5.8	+0.70		
Age	Cub	2012	0.6	0.00	12.5 (3)	0.006
	Juvenile	4710	2.2	+0.99		
	Adult	2519	2.8	+1.00		
	Old	747	2.8	+0.62		
Season	Spring	1151	2.2	0.00	18.0 (3)	<0.001
	Summer	3854	1.7	-0.34		
	Autumn	3451	1.9	-0.02		
	Winter	1532	3.5	+0.49		
Number of captures				0.05	6.2 (1)	0.013
Sex	Female	5903	1.8	0.00	2.9 (1)	0.089
	Male	4572	2.5	+0.30		
Body condition*	Poor	530	3.2	0.00	1.6 (3)	0.663
	Fair	2991	2.0	-0.40		
	Good	5890	2.0	-0.32		
	Very good	1064	2.6	-0.05		

The number of observations and percentage bite wound values are from the raw data. The effects, Wald statistic and *P* values are generated from generalized linear mixed models. Values for TB status, age, season and number of captures are from the minimum adequate model (with sex and body condition removed), where individual badger was included as a random effect (estimate ± SE = 1.01 ± 0.2). *Each individual's existing TB status and body condition were determined from the closest previous capture (see methods).

Discussion

This study investigated patterns of bite wounding in a naturally infected wild population of badgers to investigate two important aspects of disease ecology: the potential transmission of TB via bite wounding, and the behaviour of infected animals. There was no association between body location and *M. bovis* infection in bite wounds, and the detection of TB positive bite wounds at any point in an excreting badger's lifetime was significantly associated with the detection of the same positive bite wound at the badgers incident event of *M. bovis* infection (i.e. the first capture when a positive culture result was obtained). In addition, positive bite wounds were found to have no association with respiratory-related positive cultures at the incident event. A further analysis revealed that badgers that were detected as excreting *M. bovis* were significantly more likely to receive bite wounds than those that were not. The following discussion considers these results, possible mechanisms to explain them and the implications for TB transmission and disease management.
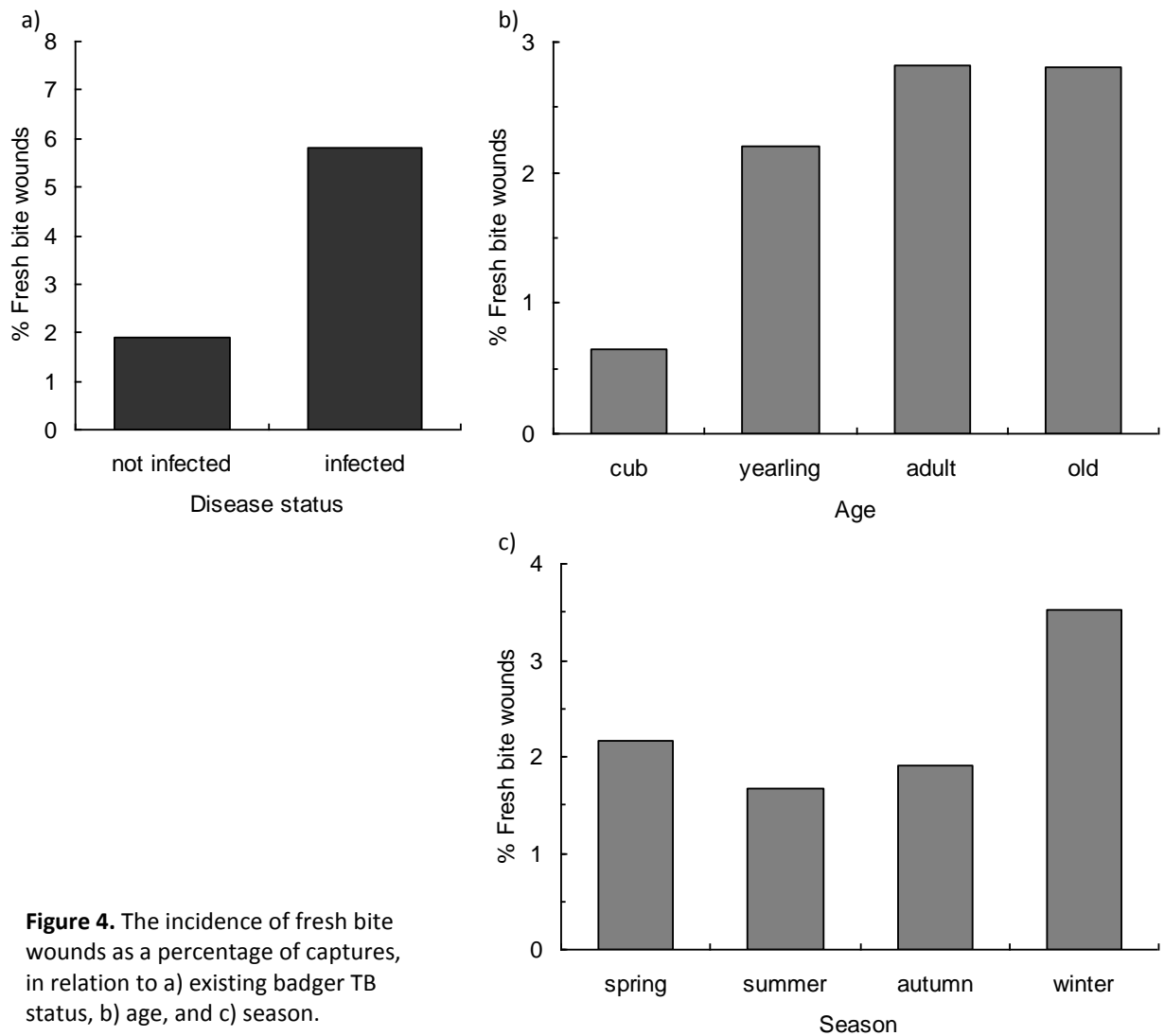
Figure 4. The incidence of fresh bite wounds as a percentage of captures, in relation to a) existing badger TB status, b) age, and c) season.

The transmission of TB via bite wounding

Positive associations between badgers having bite wounds and being infected with *M. bovis* have been documented previously in a number of studies (Gallagher *et al.* 1976; Gallagher & Nelson 1979; Cheeseman *et al.* 1989; Clifton-Hadley *et al.* 1993; Jenkins *et al.* 2008). However, whether the association is due to *M. bovis* infection transmitted as a direct result of bite wounding, or whether TB infected bite wounds could result from generalized respiratory infection, expressed secondarily at wound sites (Clifton-Hadley *et al.* 1993) is so far unresolved. The pattern of disease progression to different areas of the body is thought to be linked to the initial route of infection (Murphy *et al.* 2010) and this is the first study to analyse the TB infection status of individual bite wounds in relation to their body location in live badgers.

In this study TB infected bites were not significantly associated with any particular body location but were found to be randomly distributed across all locations (table 1). This is consistent with the idea of bite wounding as a transmission route, since culture positive wounds originating from the respiratory route would be expected to

be particularly associated with the head and neck area, which are the locations of those lymph nodes most likely to be affected by respiratory infection (Murphy *et al* 2010). In addition, badgers with culture positive bite wounds recorded at any point in their excreting lifetime were significantly more likely have the same positive wound recorded at the individual's incident event for *M. bovis infection*; there were only a small number of TB infected bite wounds in the dataset that were not present at the incident event of detection of *M. bovis* (16.7%). Of the TB positive bite wound cultures occurring at the incident event, there was no significant association with positive cultures of sputum and faeces, and 68.4% of the positive bite wounds at the incident event represented the sole culture positive sample recorded. Since with respiratory originated infection (as inferred from positive sputum and/or faecal samples) respiratory lesions are the first to develop, excretion from the respiratory tract should precede other routes (Gallagher 1998, cited in Corner 2006). As this does not appear to be the case, the direct transmission of *M. bovis* via bite wounding seems likely in these instances. Furthermore, given the high proportion of positive bite wounds that were also present at the incident event, the results are consistent with the secondary infection of bite wounds from previously established respiratory infection being uncommon. The few TB infected bite wounds arising after the incident event may have also been infected directly through the bite, rather than an expression of previously established infection, although this cannot be confirmed by the current results.

One important consideration when interpreting these results is the possibility that different culture samples may have a different level of sensitivity to the detection of *M. bovis* by culture. Hence the high level of positive bite wound swabs occurring in isolation of positive respiratory cultures could potentially be explained if the culture of bite wound swabs was significantly more sensitive at detecting *M. bovis* infection than the culture of sputum and faeces. Bite wounds can be directly swabbed on the infected area where there may be high concentrations of *M. bovis.* In contrast, aspirates of the oesophagus and trachea, and faeces samples are less likely to contain high concentrations of *M. bovis* since they are not directly sampling the site of infection.

Pathological implications of bite wounding as a transmission route

Post mortem examination of badgers has revealed similarities in the pathogenesis of disease in badgers thought to have been infected with *M. bovis* via bite wounding (Gallagher & Nelson 1979; Clifton-Hadley *et al.* 1993; Gallagher 1998, cited in Gallagher & Clifton-Hadley 2000; Jenkins *et al.* 2008). Studies commonly describe evidence of a rapidly disseminated form of tuberculosis, indicating that infection via bite wounding may typically cause severe and acute disease. Gallagher & Nelson (1979) and Gallagher (1998, cited in Gallagher & Clifton-Hadley 2000) report high occurrences of miliary lung and kidney lesions, and miliary or acute tuberculous pneumonias respectively, and both only observed certain kinds of tuberculous pneumonias in animals with tuberculous bite wounds. Similarly, Jenkins *et al.* (2008) found a higher prevalence and different distribution of lesions in badgers with recorded bite wounds (although bite TB infection status was unknown), and lesions were more likely to be in multiple areas of the body than those found in badgers

with no recorded bites. Shorter mean survival of tuberculous badgers has also been observed in cases where the initial culture was from bite wounds and abscesses compared to other samples (117 days compared to 491 days, Clifton-Hadley *et al.* 1993).

It is evident that the acute, extensive pathology of TB in badgers thought to have been infected via bite wounding is in contrast to the chronic, slow progression of disease typical of respiratory infection that badgers appear to cope relatively well with (Gallagher & Clifton-Hadley 2000). It is the extent to which infection spreads from the initial site that determines the severity and distribution of disease (Jenkins *et al.* 2008). Dissemination from the primary site of infection can occur early on in pathogenesis when infected macrophages may circulate the body via the lymphatic system or blood (Chackerian *et al.* 2002). The intradermal inoculation of captive badgers, which appears to closely reproduce the course of extensive disease seen in bite wound infected badgers in the wild, results in the spread of *M. bovis* both by the lymphatic system and haematogenously to infect organs (Pritchard *et al.* 1987). Also, experimental intradermal inoculation of badgers with large doses of *M. bovis* results in relatively rapid onset of necrosis (Pritchard *et al.* 1987). In the current study, neither bite size nor the number of bites had an effect on the TB status of bite wounds, although a higher percentage of positive bite wounds were large, compared to medium or small (table 1), consistent with the minimum infective dose for this route being relatively small. Potentially higher doses of bacilli administered via larger bite wounds could instigate more rapid disease progression.

The risks associated with bite wounding as a transmission route between badgers

The significance of bite wounding as a route of *M. bovis* transmission depends on its role in the spread and persistence of infection within and between badger populations, and the implications for spill over into cattle, which will be discussed later.

Firstly, it is important to consider which badgers within a population are most at risk of *M. bovis* infection via bite wounding. Bite wounding patterns in badger populations in southwest and southern England have been analysed using data from a long term study in Wytham Woods, Oxfordshire (Macdonald *et al.* 2004) and a combined five year study (1995 – 1999 inclusive) of badger populations in Wytham Woods, North Nibley, Gloucestershire and Woodchester Park (Delahay *et al.* 2006). These studies revealed several demographic correlates of bite wounding, some of which interacted in complex ways. The results showed that male badgers were bitten more often, obtained more severe wounds than females (Macdonald *et al.* 2004, 2008) and were more likely to have multiple bites (Delahay *et al.* 2006). This may be consistent with analysis of video-surveillance from sett entrances by Hewitt, Macdonald & Dugdale (2009) that describes evidence of possible context dependent linear dominance hierarchies within badger social groups. Females had a higher dominance rank, indicating that they initiated the most aggression and received the least aggression, whereas males generally had a low rank and received more aggression (Hewitt *et al.* 2009). Macdonald *et al.* (2004) found that the incidence of bites for male badgers also increased with the number of badgers living in

neighbouring territories, which suggests that males may receive bites as a result of territorial defence. In the current study, a higher incidence of bite wounding was also recorded in males compared to females, although this effect was not statistically significant (table 2). This could indicate differences in the badger populations when considered separately, or perhaps be due to variation in the other factors included in the statistical model (GLMM). For example, existing TB infection status and number of captures had a significant effect on bite incidence in the current study, but were not included as factors in the above analyses. Older badgers have been observed to have a higher incidence of bite wounding than cubs (Macdonald *et al.* 2004), and in the present study bite wounding also varied seasonally, peaking in winter (table 3; see also Delahay *et al.* 2006), generally reflecting breeding age and peak breeding season (mid January to mid March, Cresswell *et al.* 1992; Neal & Cheeseman 1996, cited in Delahay *et al.* 2006). Both males and females of breeding age (three to six years old) could be expected to receive more bite wounds at this time of year due to females defending young cubs or receiving neck bites from males during mating (Neal & Cheeseman 1996, cited in Delahay *et al.* 2006), and males seeking extra-group breeding opportunities in neighbouring territories (Cheeseman *et al.* 1987; Carpenter *et al.* 2005). The rate of extra-group paternity in badger social groups has been estimated by genetic studies to be around 50% at both Woodchester Park (Carpenter *et al.* 2005), and Wytham Woods (Dugdale *et al.* 2007). This indicates a significant amount of movement between social groups is taking place during the breeding season, heightening the chance of aggressive encounters and therefore increasing the opportunity for bite wounding to occur. There is evidence that a badger's movement history affects the probability of detecting wound scars (Macdonald *et al.* 2008).

No association between previous body condition and the incidence of fresh bites was found in the current study. Previous analysis of the Woodchester Park trapping data found that badgers in poor body condition had significantly more bites than those in better body condition (Delahay *et al.* 2006). Taken together, these results may indicate that body condition is not a predictor of an individual receiving a bite, but perhaps is an indirectly correlated factor. In apparent contrast, Macdonald *et al.* (2004) found that the heaviest badgers received the most bite wounds. It is evident that body condition is a complex factor which interacts with other demographic traits and is not yet fully understood.

It is not simply the risk of being bitten that determines the likelihood of infection via bite wounding, but rather the risk of being bitten by an infected individual and encountering a high enough dose of *M. bovis* for infection to be established (see Corner *et al.* 2006). *M. bovis* infected bite wounds are thought to be transmitted by diseased individuals whose mouths contain infectious sputum and saliva (Gallagher & Clifton-Hadley 2000). This could occur through the migration of bacilli from the oesophagus or trachea to the mouth through sputum, in individuals with established respiratory infection (Gallagher & Nelson 1979). Since TB is primarily respiratory orientated, any individual infected with *M. bovis* is likely to eventually develop respiratory disease, regardless of the original infection route (Clifton-Hadley *et al.* 1993; Nolan & Wilesmith 1994). *M. bovis* is commonly detected in sputum from

tracheal and oesophageal samples from infected individuals (Clifton-Hadley *et al.* 1993).

Identifying clear demographic correlates of TB distribution in badgers has proven to be challenging and the factors which determine the localised distribution of infection within badger populations are not yet fully understood (Cheeseman *et al.* 1988; Delahay *et al.* 2000). The most important risk factor for an individual to become an excretor of *M. bovis*, identified by Vicente *et al.* (2007), was the prevalence of existing excreting badgers within its social group. This is not surprising since social interactions are frequent and intimate within social groups (e.g. Böhm *et al.* 2008). The presence of infectious adult females in particular has been associated with new infections (Delahay *et al.* 2000). This includes new infections in cubs, indicating that pseudo-vertical transmission of TB may be significant, as proposed by simulation modelling (Smith *et al.* 1995), and suggested by analysis of TB prevalence and social structure in badger populations (Cheeseman *et al.* 1988). Regardless of infection status, female badgers generally have a longer lifespan than males (Cheeseman *et al.* 1988), hence, females may be particularly important in the maintenance of disease within social groups (Delahay *et al.* 2000). The incidence of TB infection is also related to movement between social groups (Rogers *et al.* 1998; Vicente *et al.* 2007). Rogers *et al.* (1998) found that increased movement between social groups preceded an increase in the incidence of new infections (as determined by the culture of clinical samples) in the following year. In addition, movement was more common in males than females (70% of females never moved, compared to 37% of males, Rogers *et al.* 1998). Subsequent analysis of the same population revealed that the positive relationship between group movement and the individual risk of becoming an incident TB case was more marked with a male biased sex ratio (Vicente *et al.* 2007). Males are also thought to be more active in defending territories (Cheeseman *et al.* 1988a), thus it seems likely that males may have more contact with badgers of other social groups than females, and may therefore have a potentially greater role in the spread of TB between social groups. Since males have generally been found to have a higher incidence of bite wounding than females (see above, Macdonald *et al.* 2004; Delahay *et al.* 2006), and the likelihood of detecting bite wound scars is associated with movement (Macdonald *et al.* 2008), this supports the potential for males in particular to be at risk of contracting and transmitting TB via bite wounding. Wilkinson *et al.* (2000) found male badgers had significantly more bite wound culture positives, whereas females gave significantly more culture positive tracheal aspirate samples, in the Woodchester Park badger population. Based on similar rates of positive culture results from bite wound swabs and tracheal aspirate samples in males and females, and assumptions that bite wounds are more likely to occur when ranging, and tracheal aspirate positives are more likely to arise from aerosol infection within the set, Smith *et al* (2001) calculate the relative rate of female between-group transmission to be 0.277 times that of males, and male within-group transmission to be 0.632 times that of females. Estimates of rates of TB transmission are a weak point in the knowledge of TB epidemiology (see Shirley *et al.* 2003), and contact rates between individuals are difficult to generalise because badger intra-group connectedness varies widely among individuals and associations between individuals vary over time (Böhm, Hutchings & White 2009).

Not all inter-group interactions in badger populations are necessarily aggressive. There are observations of neighbouring badgers meeting at latrines at the boundary between neighbouring territories without aggression (Stewart *et al.* 2002). In addition, territory may partially be maintained passively and aggressive encounters avoided by the use of boundary latrines between neighbouring social groups (Stewart *et al.* 1997).

The frequency of isolation of *M. bovis* from sputum compared to other samples, and the distribution of lesions at *post mortem* examination, suggests that overall, aerosol infection of the respiratory system is likely to be the most important for badger to badger transmission (Clifton-Hadley *et al.* 1993; Jenkins *et al.* 2008). In addition, since bite wound infected badgers may have a shorter lifespan (Clifton-Hadley *et al.* 1993), the length of time that they are infectious to other badgers may be less than for respiratory infected individuals. Continued research in the area of bite wounding is needed to determine its true significance in the epidemiology of TB in badgers.

Behaviour of culture positive badgers

The finding in the present study that badgers detected as excreting *M. bovis* are more likely to receive bite wounds than those that were not relates to the broader question of how infected individuals behave, and is relevant for all infected individuals regardless of the initial infection route. This study found that badgers with established TB infection (as detected by biological culture) subsequently had three times higher percentage bite wound incidence than uninfected badgers (table 3). The effect of this trend may be underestimated due to the inevitable inconsistencies in the trapping intervals of the wild population. Although each badger social group was trapped every three months, for some observations of individuals there was over a year between the trap event recording TB infection status and the subsequent capture detailing bite wounding incidence. During such a length of time some individuals recorded as uninfected may have become infected with *M. bovis*.

The results appear to be consistent with the hypothesis that tuberculous badgers range more widely than uninfected badgers. Movement between social groups may increase the likelihood of aggressive territorial encounters (Kruuk 1978). Hence, the detection of bite wounds has also been positively correlated with movement between social groups (Macdonald *et al.* 2008) and the number of badgers living in adjoining territories (Macdonald *et al.* 2004), particularly in males. Radio tracking and direct observation have revealed infected individuals to move significantly further than uninfected individuals (Garnett *et al.* 2005), and a similar trend has been identified, by genetic studies, in the dispersal patterns of badgers following culling (Pope *et al.* 2007). Garnett *et al.* (2005), who monitored eight same-sex pairs of excreting and non-excreting individuals, found that excreting badgers had on average 50% larger home ranges, which extended four times further into neighbouring territories, than test negative badgers. Excreting individuals also ranged over a greater proportion of their territory and foraged 65% further away from their home sett than badgers that were not excreting *M. bovis* (Garnett *et al.*

2005). In addition, anecdotal evidence describing the solitary existence and partial loss of fear of man of three severely diseased badgers, including movement into neighbouring territories by two (one with large infected bite wounds), suggests that such movement patterns may become increasingly abnormal as disease progresses (Cheeseman and Mallinson 1981). Previous analysis of the Woodchester Park badger population found no significant effect of previous exposure to TB on badger movement, where previous exposure was based on the detection of antibodies to *M. bovis* in blood samples by an enzyme-linked immunosorbent assay (ELISA) (Vicente *et al.* 2007). This suggests that there is no detectible change in movement until infection is more advanced (i.e. detected by the culture of clinical samples, Pritchard *et al.* 1986). The significant effect of existing infection status (based on the culture of clinical samples) on bite incidence in the current study of 1,717 badgers is consistent with the suggestion of Pope *et al.* (2007) that the trend describes increased movement of relatively large proportion of infected individuals, rather than a small number of badgers moving long distances.

If the association between bite wounding and badger disease status is due to movement patterns, the question of why infected individuals range more widely than uninfected individuals is raised. One potential explanation is that *M. bovis* infection induces a behavioural change in badgers. A parasite may enhance its own transmission by altering host behaviour, for example by increasing the likelihood of coming into contact with new habitat or the next susceptible host (Thomas, Adamo & Moore 2005). Pathogens may subtly alter host behaviour, without causing mortality in host populations, which may be detrimental to host persistence (Klein 2003). Such host manipulation has been identified in many species, including Norway rats *Rattus norvegicus*, which become more aggressive and increase bite wounding when infected with Seol virus (Klein, Zink & Glass 2004), and common tree shrews *Tupaia glis* which increase aggression and physical contact when infected with Borna disease virus (Sprankel *et al.* 1978). Increased movement by TB infected badgers could theoretically be an evolutionary adaptation of *M. bovis* to increase the frequency of contact with susceptible individuals in other social groups and the likelihood of aggressive territorial encounters, facilitating the spread of infection. However, since there is little evidence of parasitic manipulation of host behaviour in other bacterial infections (see Klein 2003), it may be unlikely in badgers infected with *M. bovis*.

The increased incidence of bite wounding, potentially as a result of increased ranging behaviour, may be one of the stress factors that provokes further deterioration of an excreting badger's health and reduced ability to cope with TB infection (Gallagher & Clifton-Hadley 2000). Gallagher & Nelson (1979) commonly found co-infections within badger bite wounds, sometimes resulting in extensive abscesses and death, even in the absence of TB. Phenotypic changes in hosts are complex, and interpreting whether a change in behaviour is truly adaptive for a parasite, a pathological side-effect of infection, or a host adaption to reduce the negative effects of infection, is difficult (Thomas *et al.* 2005). The abnormal behaviour of TB infected badgers could equally be a host-mediated mechanism to reduce the risk of infection to related individuals within the social group (see Loehle 1995). An

alternative explanation, suggested by Garnett *et al.* (2005), is that tuberculous individuals are forced to forage further afield, either due to being out-competed for local resources by fitter members of the social group, or to supply an increased energy demand, compensating for the drain of *M. bovis* infection on host resources. Again, the increased likelihood of excreting badgers to have bite wounds, and the potential for co-infections to occur (Gallagher & Nelson 1979) would increase the burden on infected individuals, and could be a causal factor in the reduction of fitness of diseased badgers. Consequentially, a cycle of positive feedback could develop, whereby increased bite wounding (facilitated by wider foraging behaviour) increases the stress and energy demand on a badger, which in turn provokes foraging even further afield. On the other hand, it may be that individuals that forage and range more extensively are more likely to become infected with TB (Garnett *et al.* 2005), and to receive bite wounds. Furthermore, it could be the increased likelihood of bite wounding that facilitates TB infection, via bites from other infected individuals. Deciphering whether TB infection status influences ranging behaviour or the other way around is difficult, and would probably require experimental manipulation.

An association may exist between bite wounding incidence and TB infection status independently of any observed trend in ranging behaviour. Whilst largely speculative, an additional hypothesis may explain the increased likelihood for excreting badgers to receive more bite wounds. Diseased individuals may be aggressively targeted by other group members, either indirectly via a correlated factor, such as poor body condition (Clifton-Hadley *et al.* 1993), or directly to exclude infectious individuals from group interactions and reduce the risk of further transmission. Social marginalisation via directed aggression has been observed in other social mammals, such as mandrils *Mandrillus sphinx* (Charpentier *et al.* 2004). Whilst not usually initiated via aggression, social avoidance of diseased individuals may be an important behavioural mechanism to reduce disease transmission in group living animals, but depends upon the accurate identification of infected individuals (Loehle 1995). For example, female mice have been shown to discriminate between the odours of parasitized and non parasitized males, showing preference for healthy individuals (Kavaliers & Colwell 1995). Badgers are able to distinguish between neighbours, unfamiliar individuals and self-scent via sub-caudal secretions (Palphramand & White 2007) which are highly individual-specific (Buesching *et al.* 2002). The chemical composition of sub-caudal secretions is influenced by age, body condition and reproductive success amongst other factors, so is likely to convey genuine fitness-related information about the individual to other badgers (Buesching *et al.* 2002). Such scent signals could also potentially be influenced by disease status. Marginalised infected badgers may then be identified and forced to forage further away from the main sett, and more frequently use outlying setts than uninfected individuals as observed by Garnett *et al.* (2005), and adopt a solitary existence, as noted by Cheeseman and Mallinson (1981).

The implications of culture positive badgers receiving the most bite wounds

Since badgers with established TB infection (as indicated by one or more positive culture result) are more likely to receive bite wounds than those that were culture

negative, opportunities for the transmission of TB to uninfected individuals may arise if bites are reciprocated during aggressive interactions. Bites given by diseased individuals could inoculate the receiver with *M. bovis* if bacilli are present in the saliva (Gallagher & Clifton-Hadley 2000). Such close contact between infected individuals and other badgers may also facilitate TB transmission via aerosol particles infecting the respiratory system. An additional transmission risk to other social group members may be via the ingestion of bacilli from infected bite wounds during grooming, although the risk of infection by this route may be small due to the low pH of gastric secretions potentially forming an effective barrier against *M. bovis* (Giannella *et al.* 1973). In addition, the small proportion of infected bites that were not present at the incident TB event in this study is consistent with secondary expression of respiratory infection through bites being relatively infrequent. Bite wounding in infected individuals may aid quicker deterioration of general health, thereby reducing the amount of time infectious individuals pose a transmission risk. However, before death such individuals may excrete high amounts of *M. bovis*, which could pose a particular risk to cattle (Gallagher & Clifton-Hadley 2000).

TB transmission from badgers to cattle: the implications of bite wounding transmission between badgers and the increased likelihood of infected badgers being bitten

The primary route for TB transmission from badgers to cattle is considered by some to be via inhalation of *M. bovis* through grazing on contaminated pasture (Hutchings & Harris 1999; Pollock & Neil 2002). Badger urine, and faeces to a lesser degree, can contain large numbers of bacilli (up to 300,000 ml^{-1} in urine, MAFF 1979), which can remain infectious in the environment (Courtenay *et al.* 2006), and any excretion from an infected badger containing *M. bovis* is a potential unquantified risk. Transmission may also occur through the consumption of contaminated material (Pollock & Niell 2002), which could happen during unselective feeding on cattle feed that infected badgers have defecated in (Garnett, Delahay & Roper 2003; Tolhurst *et al.* 2009). Cattle have been observed to graze at badger latrine sites, which they would normally avoid, when there is competition for pasture (Hutchings *et al.* 1997). The detection of viable *M. bovis* at badger latrines and setts has been strongly linked to the frequency of *M. bovis* excretion by infected badgers (Courtenay *et al.* 2006). Badgers with progressed disease can excrete large amounts of bacilli and are therefore thought to pose the highest risk to cattle (Gallagher & Clifton-Hadley 2000). Since an infected badger's ability to cope with TB may be compromised by the presence of bite wounds, which can cause a significant level of additional stress on an infected individual's immune system and resources (Gallagher & Clifton-Hadley 2000), the progression of disease may be accelerated and the transmission risk that an infected badger poses to cattle increased. A similar risk may be posed by badgers infected via bite wounding, which also typically experience severe and acute disease (Gallagher & Nelson 1979), although their potentially shorter lifespan (Clifton-Hadley *et al.* 1993) may mean that the opportunity to excrete *M. bovis* occurs over a shorter period of time.

Direct contact between badgers and cattle may also be important for TB transmission (Tolhurst *et al.* 2009). Contact can occur at farm buildings (Garnett,

Delahay & Roper 2002; Tolhurst *et al.* 2009), feed troughs (Garnett *et al.* 2003), and on pasture (Böhm *et al.* 2009). Although contact rates vary greatly between individuals and may be relatively infrequent (Böhm *et al.* 2009), this provides an additional opportunity for aerosol transmission of *M. bovis.* Diseased badgers may be more motivated to feed from artificial resources, such as cattle feed troughs (Garnett *et al.* 2002), particularly if they have a higher energy demand or cannot compete for the optimum foraging patches, as suggested by Garnett *et al.* (2005). Infected badgers were discovered on farms with persistent TB in cattle in the 1970s (Muirhead *et al.* 1974, cited in Ward, Judge & Delahay 2010), and observations by Cheeseman and Mallinson (1981) suggest that badgers with advanced disease, such as is seen in badgers thought to have been infected via a bite (Gallagher & Nelson 1979), may spend more time around farm buildings and avoid cattle less than uninfected badgers. TB infected brushtail possums *Trichosurus vulpecula* reduce their avoidance of cattle on pasture (Morris & Pfeiffer 1995, cited in Ward *et al* 2010). This results in increased direct contact between the species as cattle investigate diseased possums, and consequentially is thought to be the main transmission route between possums and cattle in New Zealand (Morris & Pfeiffer 1995, cited in Ward *et al* 2010). The abnormal movement patterns of TB infected badgers (Cheeseman & Mallinson 1981; Garnett *et al.* 2005), which may be enhanced by the incidence of bite wounding, could be important for transmission to cattle, particularly if such changes in behaviour facilitate more time spent near farm buildings and feed stores (Tolhurst *et al.* 2009). Open infected bite wounds on infected badgers pose a further potential source of infection during direct contact, and male badgers, which may be more likely to have bite wounds (Macdonald *et al.* 2004; Delahay *et al*. 2006), have been more frequently located in buildings than female badgers (Tolhurst *et al.* 2009).

Implications for management to reduce TB in badgers and cattle

Given that the likelihood of detecting bites among badgers is positively associated with movement history (Macdonald *et al.* 2008), the transmission of TB between badgers via bite wounding may potentially be increased by any management technique that facilitates increased movement in badger populations. Field studies indicate that management by culling may cause increased immigration into culled areas, disruption of territoriality, and increased ranging and mixing between badger social groups (Carter *et al.* 2007), which may elevate the risk of bite wounding transmission between badgers. This could potentially have contributed to the observed increase in TB in cattle following some culling programmes (see Donnelly *et al.* 2007), and should be taken into consideration in the future use of culling as a management tool for disease control.

Since bite wound transmitted TB infection appears to accelerate the progression of disease in badgers, the occurrence of behavioural changes leading to increased ranging behaviour, possibly including increased visitation to farm resources, may potentially be more frequent and occur earlier in bite infected individuals compared to those with respiratory originated infection. This, combined with the increased likelihood of excreting badgers to have fresh bite wounds (a potential source of *M. bovis* bacilli), emphasises the importance of farm husbandry techniques to limit the

opportunities for direct and indirect contact between badgers and cattle, such as the exclusion of badgers from farm buildings, including feed stores (see Ward *et al.* 2010).

Conclusions and implications for disease management

This study found *M. bovis* infected bite wounds to be randomly distributed across all body locations, and positively associated with the incident event of TB infection in badgers, with no association to positive respiratory-related cultures. This demonstrates the potential for bite wounding as a true route of TB transmission between badgers, and for aggressive interactions to be important in the transmission of bacterial infections. Whilst aerosol infection of the respiratory system is accepted as the primary route of TB transmission between badgers, indications that infected individuals show abnormally increased ranging behaviour (Cheeseman & Mallinson 1981; Garnett *et al.* 2005), combined with the current results that infected individuals are more involved in aggressive encounters (shown by bite wounding incidence), may suggest that bite wounding transmission could be important for the spread of TB between unfamiliar social groups.

Both bite wound infected individuals and infected individuals with bites may be important for TB transmission to cattle due to potentially high levels of excretion, and should be taken into consideration when planning a TB management programme.

Acknowledgements

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