Epidemiological dynamics of *Mycobacterium bovis* and population suppression in badgers (*Meles meles*)

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Declarations

All work presented in this thesis is my own, under the supervision of Professor Christl Donnelly and Professor Rosie Woodroffe. Any other work that is not my own has been appropriately referenced.

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Abstract

Bovine TB in cattle is a major economic problem in the UK, costing the government approximately £100m a year. Badgers are a wildlife host of the infection that causes bovine TB, and there is strong evidence that they transmit the infection to cattle. Understanding the ecology and epidemiology of infected badger populations is therefore crucial for implementing disease management strategies relating to badgers.

Genetic and phenotypic data of badgers captured during a large-scale field trial of repeated, widespread badger culls were used to assess the importance of parental roles on the impacts of badger culling. Further ecological and epidemiological dynamics were investigated through developing a stochastic simulation model of an infected badger population.

An estimated 72.8% of badgers were captured during initial culls of the trial, and an estimated 57.8% during follow-up culls, based on badger parentage assignments. Further analyses of these parentage assignments revealed evidence of a genetic predisposition in infection susceptibility from parents to cubs, but no evidence of a significant infection transmission route from mothers to their young, dependent cubs. There was also no evidence that badger welfare was compromised during the trial through not capturing dependent cubs of culled mothers. Analysis of a simulation model found that moderate levels of disease-induced mortality in an infected badger population could significantly reduce the size of badger social groups with a higher prevalence of infection.

The impact of these findings relating to potential disease management strategies is discussed, together with suggested directions for future research.

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Chapter 1 – Introduction

Bovine tuberculosis, cattle and badgers (*Meles meles*)

Bovine tuberculosis (TB) is an infectious disease found in many mammalian species, and is caused by the bacterium *Mycobacterium bovis*. The bacterium is known as having one of the broadest ranges of hosts of all pathogens, and has been reported to have caused disease in cattle, bison, buffaloes, marsupials, equines, camels, pigs, sheep, goats, deer, antelopes, elephants, cats, dogs, foxes, mink, badgers, moles, ferrets, rats and primates (Francis, 1947, Francis, 1958, Lepper and Corner, 1983, MAFF, 1984). The isolation of tubercle bacilli from both human and bovine sources was first reported in 1882 (Koch, 1884). *M. bovis* transmission can occur via the aerogenous and alimentary routes, as well as through faeces, urine, sputum and pus (Morris *et al.*, 1994). *M. bovis* is also known to be able to cross the species barrier, including to humans (Morris *et al.*, 1994), however human-to-human transmission is very rare (Evans *et al.*, 2007).

Bovine TB affects livestock in many countries (Brooks-Pollock *et al.*, 2013). In the UK there has been a steady increase in bovine TB incidence levels in cattle herds for several decades (DEFRA, 2014), costing millions in disease management measures. As well as cattle, badgers (*Meles meles*) are a known to be a host species of *M. bovis* infection. There is considerable evidence that *M. bovis* is transmitted between cattle and badgers (Bourne *et al.*, 2007, Krebs *et al.*, 1997, Little *et al.*, 1982), and that badgers therefore play a significant role in infecting cattle with *M. bovis*.

The cost of bovine TB in England

Bovine TB in cattle is a huge economic problem in England, costing the government in the region £500m over the last decade; furthermore, this figure is predicted to double to £1bn over the next decade if the problem does not improve, with the cost in 2014 alone expected to be over £100m (DEFRA, 2014). The main components of these costs are for farmer compensation for a herd breakdown costs, and research. The average cost of a cattle herd breakdown due to bovine TB in England is approximately £30,000, with the farmer bearing £10,000 of this cost, and the government contributing £20,000 (DEFRA, 2014).

As well as the costs of dealing with the problem of cattle herd breakdowns outlined above, there is also a substantial amount of scientific research into the issue. Over the 2013-2014 financial year more than £8m was invested by the government to tackle the disease in cattle (DEFRA, 2014). The core areas of active bovine TB research include: badger and cattle vaccine development; research into the feasibility of different culling and vaccination practices; improving diagnostic tests used in badgers and cattle (for example, developing a live *M. bovis* test in badgers); and epidemiological and socioeconomic studies.

The development of an effective TB vaccine for badgers might negate the need to consider using badger culling as a disease management strategy. The current government has pledged to invest £24.7m on the development of vaccines alone by 2015 (since they came into government in 2010, DEFRA, 2014). Two types of vaccine to tackle *M. bovis* in badgers are currently being researched: an injectable vaccine and an oral vaccine. In March 2010 the first injectable vaccine for *M. bovis* in badgers, Bacillus Calmette-Guérin (BCG), was licensed. Scientific support for licensing this vaccine came about from studies showing that it could reduce the progression, severity and excretion of *M. bovis* infection in badgers (Chambers *et al.*, 2010). In July 2010 the government commenced a 5 year 'Badger Vaccine Deployment Project' in an area of Stroud in Gloucestershire to test the practicalities of delivering this injectable vaccine to badger populations. The project was also designed to train volunteers who wished to gain a license to vaccinate badgers. There is also ongoing work towards developing an orally administered badger TB vaccine, and although oral vaccines for badgers have been shown to work in principle (Corner *et al.*, 2010, Murphy *et al.*, 2014), demonstrating that an oral vaccine can be used effectively as a disease management strategy over a large-scale operation is thought to be some years away.

As well as research into developing a vaccine for badgers, there is a large amount of work being done to develop a vaccine for cattle. As of 2014 there is no vaccine licensed for use on cattle in the UK, however progress in this area could be a crucial step in managing and eradicating the disease in cattle. Although a BCG cattle vaccine does exist, it does not fully protect cattle, and so a vaccinated bovine could become infected with TB if exposed to *M. bovis* infection (Waters *et al.*, 2012). However the tuberculin TB skin test (the standard test used to test cattle for TB) cannot differentiate between infected and uninfected vaccinated cattle (Whelan *et al.*, 2011). It is therefore against EU law to use this vaccine on cattle. Consequently, there are also parallel research efforts to develop a test that is able to distinguish between infected animals and uninfected vaccinated cattle, known as a DIVA

(Differentiating Infected from Vaccinated Animals) test, which if licensed could lead to a relaxation on the EU restriction on using this BCG cattle vaccine. In January 2013 the EU commissioner for health and consumer policy Tonio Borg set out a proposed timeline of cattle TB vaccination in a letter to DEFRA minister Owen Paterson (DEFRA, 2014), estimating that nationwide use of a cattle vaccine might be feasible within 5-10 years.

Testing for bovine TB

In England cattle are subjected to a test and slaughter programme. Herds at high risk of TB, most of which are in the west and southwest of England, are tested annually for TB; herds at low risk of TB are tested every four years.

The first stage of the testing procedure uses the single intradermal comparative cervical tuberculin (SICCT) test, more widely referred to as the tuberculin skin test. If at least one animal produces a positive test result, then the herd's Officially Tuberculosis-Free (OTF) status is suspended and test-positive animals are slaughtered; this is termed a "herd breakdown". Movement restrictions are then applied to the herd until the remaining animals in the herd pass two subsequent tests 60 days apart (DEFRA, 2014).

A second test, the gamma-interferon (g-IFN) blood test, is more sensitive (but less specific) at detecting TB in cattle than the tuberculin skin test described above (Schiller *et al.*, 2011). In England it is used compulsorily on animals from a confirmed herd breakdown in a low risk area that gave a negative tuberculin skin test (DEFRA, 2014). It is also used on a discretionary basis on animals from a confirmed herd breakdown in a high risk area that give a negative tuberculin skin test, and occasionally in circumstances of suspicious initial test results (e.g. potentially fraudulent testing procedures).

Attempts to develop a live test for *M. bovis* infection in badgers have resulted in tests with *M. bovis* sensitivity estimates of between 50-80% (Drewe *et al.*, 2010). If a more sensitive live badger TB test was developed, then the possibility of implementing a policy based around the selective culling of badgers (in which only infected badgers would be culled) in England might be available, however it is inconclusive whether this would negate the detrimental effects of non-selective badger culling on cattle TB levels from perturbation (Bielby *et al.*, 2014).

Badger culling in England

On 14th December 2011, the government confirmed that two pilot culls would take place in the areas of West Gloucestershire and West Somerset in 2012 over a six week period. These were designed to determine whether culls allowing the free shooting of badgers could attain a culling efficacy of at least 70% in the culled areas, and whether free shooting badgers could be carried out humanely and safely. If these targets were achieved in the pilot culls then it was planned that the scheme would be rolled out across England. The method of cage-trapping badgers was also permitted during these culls.

The two pilot culls had been due to begin in the early summer of 2012, however after several delays the culls kept being pushed further back. In order to maximise the chance of achieving a badger culling efficacy of at least 70%, the pilot culls needed to be carried out before the late Autumn and Winter months, during which time badgers are less likely to emerge from their setts. With the culls still not having started by October 2012, DEFRA minister Owen Paterson announced that the culls would be postponed until 2013.

The pilot culls did eventually take place in 2013, with the six week culling period beginning on 13th August. However as the culls progressed, it was clear that the target of culling at least 70% of the badgers in the culling areas was not going to be met within the six week time-frame. Therefore a controversial decision to extend the time-frame of both the pilot culls to nine weeks was taken, in order to try and attain a culling efficacy of at least 70%. The resultant estimates of the culling efficacies of these pilot culls were between 43.0% and 55.7% (95% confidence interval [CI]) in West Gloucestershire, with between 29.2% and 42.2% culled by free shooting; corresponding estimates in West Somerset ranged between 37.0% and 50.9% (95% CI), with between 14.2% and 25.3% culled by free shooting (DEFRA, 2014). The pre-defined target for the two pilot culls relating to humaneness was that at least 95% of badgers culled by the method of free shooting must have died within five minutes of being shot, in order to avoid significant suffering to the badger. The analyses from these pilot culls estimated that between 7.4% and 22.8% of the badgers culled by free shooting took longer than five minutes to die, and so the culls did not attain the pre-defined target relating to humaneness (Munro *et al.*, 2014).

Despite extensive criticism surrounding the failure to attain the targets for these pilot culls relating to badger culling efficacy and humaneness, the government announced in April 2014

that it would continue with the culls in the same two pilot areas for 2014-2016, with no plans to assign an independent panel to monitor these culls.

Bovine TB elsewhere in the UK

Bovine TB is also a serious animal health problem in Wales, with thousands of new cattle TB incidents every year. In 2005, responsibility of animal health and welfare issues in Wales was handed to Welsh ministers. Following a 2007 report providing evidence of an association between bovine TB in cattle and badgers (WAG, 2014), the Welsh Assembly Government (WAG) decided in October 2009 to authorise a pilot badger cull in North Pembrokeshire as part of Wales' TB Eradication Programme; however this was not planned to take place until April 2010. In response to this decision the Badger Trust sought a judicial review to stop the culling on the basis that it would not eliminate or substantially reduce cattle TB incidence, as required by the Animal Health Act 1981. However the High Court ruled in April 2010 that the culling plan was lawful. The Badger Trust was granted the right to appeal in June 2010, and was successful in persuading the Court of Appeal to quash the original decision to allow badger culling. After a change in government leadership in 2011, the new WAG authorised a five year badger vaccination scheme that began in May 2012 in western parts of Wales (known as the 'Intensive Action Area'). In 2013 the number of new bovine TB incidents in Wales fell by 22.4% from 1,119 in 2012 to 868, and the number of cattle slaughtered as a result of TB controls fell by 34.4% from 9,308 in 2012 to 6,102 in 2013 (WAG, 2014).

Northern Ireland has adopted a different approach to the issue of TB in badgers. In June 2014 a five year research project began, investigating the effects of selective badger culling in Northern Ireland, known as the Test and Vaccinate or Remove (TVR) project. Taking place in a 100km^2 area around Banbridge, this involves trapping and testing badgers for *M. bovis*; if they test negative then they are vaccinated, if they test positive then they are killed. However there is evidence that even selective badger culls, in which fewer badgers may be culled than in non-selective culls, may result in increasing levels of bovine TB in cattle (Bielby *et al.*, 2014, Donnelly *et al.*, 2006, Smith *et al.*, 2013). Furthermore, the low sensitivity of live badger tests (Chambers *et al.*, 2008, Drewe *et al.*, 2010) would result in some infected badgers testing negative for *M. bovis* and being released.

Scotland has been Officially Tuberculosis Free (OTF) since 2009, and thus bovine TB is not considered a major threat to animal health.

Bovine TB outside the UK

There are many areas in the world where *M. bovis* infection causes a threat to livestock, however there are two well documented cases involving a wildlife host of infection: badgers and cattle in the Republic of Ireland, and possums with cattle and deer in New Zealand.

The practice of using snares to cull badgers in Ireland has been implemented for many years, and the number of herds under TB restrictions due to a bovine TB incident in Ireland has fallen over the last decade (DAFM, 2014). However, Ireland also operates a more frequent cattle TB testing policy in low risk areas compared to England (annually, compared to every four years in England) (White *et al.*, 2013). Furthermore, badger population densities in Ireland are much lower than in England (Palphramand *et al.*, 2007), and any parallels made between the effects of badger culling on bovine TB levels in Ireland and England are potentially tenuous.

In New Zealand, brush-tailed possums are the main wildlife hosts of *M. bovis* infection (Karlson and Lessel, 1970) and were long known to be the main wildlife source of *M. bovis* transmission to cattle and deer herds (Caley *et al.*, 1999, Coleman and Caley, 2000). However, intensive control measures on possums saw the incidence of TB in livestock herds decrease dramatically (Animal Health Board, 2005). A major difference between the bovine TB problem in New Zealand and the one in the UK is that possums are a non-native conservation pest in New Zealand, and so there are fewer issues in using strategies that involves culling possums, whereas badgers are a protected species in the UK. The method of possum population control used in New Zealand (dropping poisoned bait) was still a contentious issue, however an oral vaccine for possums infected with *M. bovis* was later developed (Aldwell *et al.*, 2003, Buddle *et al.*, 2006).

Other occurrences of bovine TB problems around the world have been seen in white-tailed deer in the USA (Schmitt *et al.*, 1997), elk in Canada (Lees, 2004), South African buffalo (De Vos *et al.*, 2001), and wild boar and red deer in Spain (Vicente *et al.*, 2007).

The behaviour and ecology of badgers (*Meles meles*)

European badgers (*Meles meles*) are members of the weasel (*Mustelidae*) family, and are found in most European countries. In Britain many badgers reside in deciduous woodland areas or copses, however they are also known to live in a wide range of habitats; this adaptability is a key to their success as a species, which is demonstrated by their wide

distribution across Europe (Neal and Cheeseman, 1996). As mentioned earlier, badgers live in underground setts, which are networks of tunnels that often have between 3-10 entrances, each of which are approximately 300-350mm in diameter (Neal and Cheeseman, 1996). The ideal habitat for a badger sett would consist of sandy, well-oiled soils (Macdonald *et al.*, 2004).

In England badgers are densely populated in the south, southwest and west of the country, and in Wales they are more predominantly found in the southwest (Cresswell *et al.*, 1990). As badgers are known to be crepuscular during the summer months and nocturnal during the winter months (Neal and Cheeseman, 1996), as well as being fossorial, estimating badger abundance is difficult. Cresswell *et al.* (1990) estimated there to be approximately 216,000 adult badgers in Britain, whilst a separate estimate found a higher value of 250,000 adult badgers living in Britain (Neal and Cheeseman, 1996); a further 38,000 badgers were estimated to be living in Northern Ireland (Feore, 1994). Badger social group densities can be up to 0.6-0.7km^{-2}, however more common group densities in Britain are in the region of 0.3-0.4km^{-2} (Cresswell *et al.*, 1990).

Badgers are social animals, and typically live in groups of up to 10 individuals, however natural social groups of up to 35 individuals have been recorded in Woodchester Park, Gloucestershire (Neal and Cheeseman, 1996). In undisturbed populations, badger social groups usually defend well defined, non-overlapping territories, and each group will often have more than one sett within a territory. Social group size can vary greatly depending on food availability, as well as other climatic and environmental factors (Kruuk and Parish, 1982). As badgers are better suited as general foragers rather than specifically as hunters, they are omnivorous and can have a hugely varied diet (Kruuk, 1978). Their main food source in the UK is earthworms, however they will also eat insects, general arthropods, fruit, vegetation, small mammals (voles, mice, rats, moles, hedgehogs, rabbits, hares), cereals and seeds (Neal and Cheeseman, 1996).

Cubs are usually born between mid-January and mid-March, with the majority being born in February (Neal and Cheeseman, 1996). Mating can occur at any time of the year, however delayed implantation in the female means that all cubs are born during this season (Canivenc and Bonnin, 1979, Fischer, 1931). Cubs spend around the first 8 weeks in an underground sett before emerging for the first time; however they still remain dependent on their mother's milk for their first 12 weeks of life, and often longer (Neal and Cheeseman, 1996). Cub

mortality is high, with estimates of up to half of cubs not surviving their first year of life, whilst adult annual mortality is much lower (estimated to be 21%, Rogers *et al.*, 1997). Other than humans, badgers do not have any natural predators in the UK, and badgers that are not killed by humans (either through a road traffic accident or by culling) are thought to often die in their underground sett (Neal and Cheeseman, 1996).

Aims and overview of thesis

In this thesis I address some of the major gaps in the understanding of badger ecology in relation to the epidemiology of *M. bovis* in badger populations. The majority of the data used in this work originate from the proactive culls of the Randomised Badger Culling Trial (RBCT), a large-scale badger culling trial that took place in England between 1998 and 2005 (details of which are outlined in Chapter 2 as part of an overview of the history of bovine TB in the UK and the Republic of Ireland).

In Chapter 3 I estimate the parentage of cubs taken during the proactive culls of the RBCT using genetic data of adults and cubs. For each cub, a probability that its mother/father was in the dataset (i.e. had been culled) was estimated, and these values were used to infer the proportion of adults that were captured during the RBCT proactive culls. Previous estimates of badger population reduction during the RBCT (Woodroffe *et al.*, 2008) were used as a licensing criterion for the 2013 pilot badger culls in England, stating that at least 70% of badgers must be culled in the two pilot areas (DEFRA, 2014). However Woodroffe *et al.* (2008) did not directly measure badger abundance reduction, but estimated the reduction of measures highly associated with badger abundance, including badger latrine density (73%), active badger burrow density reduction (69%) and badger road kill density reduction (73%).

In Chapter 4 I use the parentage data generated in Chapter 3 to investigate the association between parent and cub *M. bovis* infection status. It has been hypothesised that mothers transmitting *M. bovis* infection to their dependent cubs through close contact in their first few weeks of life, known as pseudo-vertical transmission, could contribute to the persistence of *M. bovis* infection in badger populations (Anderson and Trewhella, 1985, Morris *et al.*, 1994). Furthermore, this work also tests the hypothesis that cubs may inherit a predisposition in susceptibility to *M. bovis* infection by assessing father-cub infection association, as well as mother-cub infection association. Almost half of fathers are from outside the social group of their cubs (Dugdale *et al.*, 2007), and so any positive father-cub infection association would

be likely to be due to a genetic factor as opposed to an infection transmission event related to their relative proximity.

Chapter 5 makes further use of the parentage data generated in Chapter 3 to assess whether it is likely that cubs of culled mothers were missed during the RBCT whilst they were still dependent on their mothers. During the RBCT a 'closed season' running from February-April inclusive was designated as a time of no culling so as to avoid culling the mothers of dependent cubs. A considerable majority of cubs (estimated at 76%) are born between mid-January and mid-March (Neal and Cheeseman, 1996), and during these months cubs are at risk of starvation if their mother is not able to provide them with milk (Woodroffe *et al.*, 2005). If cubs were still dependent on their mothers after the closed season has ended, this could be a potentially serious issue relating to the humaneness of badger culling. Using high-probability mother-cub parentage assignments, the mean number of cubs per mother was calculated and compared against expected values of mother:cub ratios based on cub survival and trappability rates estimated from other badger populations.

The final data chapter of this thesis, Chapter 6, investigates a potential mechanism to explain an observed relationship between badger social group size and *M. bovis* prevalence. Woodroffe *et al.* (2009) found a significant negative association between group size and infection prevalence in badgers culled during the RBCT, contrary to previous predictions regarding badger TB dynamics (Anderson and Trewhella, 1985, Smith *et al.*, 1995). A strong candidate hypothesis explaining this association was that disease-induced mortality might suppress group size, resulting in infected groups being depleted of individuals. To test this, an individual-based stochastic model which simulated badger population dynamics and *M. bovis* infection was developed, and the extent to which disease-induced mortality was added to baseline mortality was varied.

The final chapter summarises the results of this thesis, and discusses their wider implications. The findings I present in this work offer a balance in ecological and epidemiological emphases, as well as a mix of both theoretical insights and applied results that can be used to help inform policy. I also discuss how further scientific understanding in these fields could be gained if new data were available, and how this may further impact policies relating to badgers and bovine TB.

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Chapter 2 – History of bovine TB in the UK and the Republic of Ireland

History of bovine TB in the UK

In the UK bovine TB has been a problem for many years. During the late 19th and early 20th century there was widespread concern over the dangers of bovine TB to human health through infected meat and milk. In the 1930s over 2,500 human deaths per year were attributed to bovine TB (Bourne *et al.*, 2007), and an estimated 40% of cattle were reported to be infected with *Mycobacterium bovis* (Proud, 2006). To tackle the issue, the Gowland Hopkins Committee wrote a report (Economic Advisory Council, 1934) that initiated three major steps towards eliminating bovine TB in both humans and cattle: the pasteurisation of milk, meat inspection, and elimination programmes based on the slaughter of cattle that tested positive for bovine TB. Efforts to reduce bovine TB levels in cattle through the slaughter of infected livestock began in 1935 as a voluntary programme (Collins and Grange, 1983), but was halted due to the outbreak of the Second World War in 1939. When the programme re-started in 1950 it was made compulsory to slaughter cattle that tested positive for TB (Zuckerman, 1980). The 1950s and 1960s saw a drastic reduction in bovine TB incidence rates, with the percentage of infected herds in the UK following a downward trend from 18% in 1950, to 3.5% in 1960, 1% in 1965, and continuing to 0.18% in 1979 (Collins and Grange, 1983). These figures suggested that bovine TB had been all but eliminated in British cattle, however this decreasing incidence pattern was not being followed in regions of South West England (Bourne *et al.*, 2007), and prompted a field study by the Ministry of Agriculture, Fisheries and Food (MAFF) in West Cornwall in 1970, where the bovine TB incidence rate was particularly high. The concluding report (Richards, 1972) primarily blamed poor farm biosecurity, in particular the condition of the bordering fences between farms.

Whilst bovine TB was a well-documented disease of cattle during this time, little was known about the disease in other animals. In 1959, *M. bovis* was first identified in a badger (*Meles meles*) in Switzerland (Grange and Collins, 1987), and in 1970 *M. bovis* infection was identified in the brush-tail possum (*Trichosurus vulpecula*) in New Zealand (Eckdahl *et al.*, 1970). Further investigation found that both badgers and possums were effective maintenance

hosts of *M. bovis* relative to most other animal hosts of the infection (Morris *et al.*, 1994). It was not until 1971 that the first badger in the UK was identified as having *M. bovis*, and this marked a significant turning point in the history of bovine TB control measures in the UK. In 1972 the MAFF began an inquiry into the relationship between badgers, cattle and bovine TB, and in 1973 concluded that action needed to be taken with badgers in order to reduce the threat of the disease to cattle (Bourne *et al.*, 2007).

In 1974 the MAFF began a programme of badger culling in South Dorset, initially with cage-traps. However due to the low number of badgers caught (33 over a nine month period, Thompson, 1978) it was decided in 1975 that it would be more effective to gas badger setts with cyanide. This decision generated much controversy, with conservationists strongly opposed to the policy, but conversely met with great support from the farming community; the issue was consequently labelled "The Great Badger Debate" (Grange and Collins, 1987). The gassing took place between 1975 and 1982 in 166 areas throughout the southwest of England, averaging 7km^2 per area (Wilesmith, 1986). In certain regions the programme appeared to be effective in terms of reducing bovine TB levels, with an associated 10 year period of no confirmed cattle herd breakdowns in an area near Thornbury in Gloucestershire (Clifton-Hadley *et al.*, 1995). Also in 1975, the MAFF set up a long-term research programme in Gloucestershire (Zuckerman, 1980) to collect and analyse data relating to the population and disease dynamics of *M. bovis*-infected badger populations (this included population densities at individual and social group levels, dispersal, mortality, and prevalence of *M. bovis* infection), and to publish annual reports of their findings. Although analyses of the data collected from this programme gave significant insight into the distribution of *M. bovis* in badgers and the role of social structure, it was concluded that more information on the subject was required in order to adequately deal with the issue of badgers and bovine TB (Cheeseman *et al.*, 1981, Cheeseman and Mallinson, 1981, Cheeseman *et al.*, 1988, Thornton, 1988).

Pressure to stop the gassing of badger setts from those concerned about the effectiveness and humaneness of the method was increasing, and in 1979 the MAFF ordered a review of the policy to be undertaken by Lord Zuckerman, during which time the gassing was temporarily halted until the report was published (Zuckerman, 1980). In the resultant report Lord Zuckerman outlined the lack of knowledge and understanding of the speed at which badgers die from cyanide gas, and considered two chemical alternatives to cyanide for the gassing of badger setts: carbon monoxide and the nerve gases. However, he deemed both to be

unsuitable for such a purpose: the former not being toxic enough, and the latter being too toxic to be used safely. Zuckerman also highlighted the importance of ensuring that as many badgers as possible were in their residing main sett when the gassing takes place, so that badgers were concentrated in as few places as possible when gassing took place; this was argued to be during late autumn and winter.

The report also discussed several other control methods, including free shooting, snaring and cage-trapping. Free shooting and cage-trapping were both deemed impractical in the report, however this was likely to have been in comparison to the much cheaper alternative of gassing badger setts that had been used for the previous five years. Zuckerman argued that snaring, if performed by professionals, could be performed humanely. However he was keen to point out the dangers of inhumane snaring if performed by amateurs, and together with strong public disapproval for using snares as a method of capturing badgers, was not considered a viable option.

The Zuckerman report concluded that there was a link between badgers, cattle and bovine TB, and moreover that the threat of the disease being passed from badgers to cattle was great enough to justify the gassing of badger setts to continue (Zuckerman, 1980). However the report also suggested investigating the efficacy and humaneness of the method of gassing badger setts, and recommended that the government's Chemical Defence Establishment design experiments to assess the feasibility of humanely gassing badger setts.

The results of these experiments showed that there were serious doubts over whether badgers that died from cyanide gas had died quickly, and thus humanely (Dunnet *et al.*, 1986). In light of these new findings the government changed the method of badger culling from the gassing of setts to cage-trapping and shooting (Dunnet *et al.*, 1986). That was not to say that cage-trapping was a widely accepted policy, with later research on cage-trapping finding potential drawbacks on the use of cage-trapping on badger welfare (Woodroffe *et al.*, 2005).

In 1982 the 'clean ring' strategy of badger culling was implemented: badgers in known territories near confirmed herd breakdown sites were trapped and tested; if at least one badger tested positive, then the culling operation extended to all adjacent badger social groups; this process continued until no infection could be identified in a "clean ring" of badger social groups around the confirmed herd breakdown site (Bourne *et al.*, 2007).

As more evidence supporting the association between badgers, cattle and bovine TB mounted (with significant findings from epidemiological studies in Dorset (Little *et al.*, 1982a, Little *et al.*, 1982b, Little *et al.*, 1982c, Wilesmith *et al.*, 1982) and East Sussex (Pritchard *et al.*, 1986, Wilesmith *et al.*, 1986a, Wilesmith *et al.*, 1986b, Wilesmith *et al.*, 1986c)), pressure to use cheaper and more effective methods of population control resulted in a further review of the situation in 1984. This was chaired by Professor George Dunnet, with the aim of discussing how the problem of bovine TB and badgers had progressed since Zuckerman's report in 1980. The Dunnet report held the method of cage-trapping in a more favourable light compared to Zuckerman's report, arguing that the resultant badger carcasses could be used for scientific investigation.

The authors were keen to stress that there were many gaps in the understanding of the epidemiology of *M. bovis* in badgers, and even hypothesised the translocation of badgers (removing a group of badgers from one area to another) as a reason for the wide distribution of the problem of badgers and bovine TB. However they did reiterate that there was significant evidence pointing towards a link between badgers, cattle and bovine TB, and thus could not justify a cessation in badger culling. However the report also concluded that gassing had no significant effect on lowering the number of herd breakdowns, and said the reduction in incidence shortly after the introduction of gassing could have also been credited to a change in policy around the same time which restricted cattle imports from Ireland, as well as an improvement in the tuberculin skin test (similar reductions in incidence occurred in Ireland around the same time, where there was no culling). The lack of evidence for the effectiveness of gassing, combined with the high cost of the clean ring strategy, led to the introduction of a new culling strategy known as the 'interim' strategy (given its name as this strategy was considered an interim while waiting for the development of an effective live test). Under this strategy a badger cull was performed in an area of a confirmed herd breakdown if there was sufficient evidence to attribute that breakdown to *M. bovis* infection in badgers. This meant not having to trap and test badgers as in the previous clean ring strategy, saving time and expense. Furthermore, culling was not extended to contiguous badger social groups as in the clean ring strategy. This strategy was initially designed as an interim in the hope that it would be a short-term solution until a live diagnostic test for badgers was developed, however this strategy was virtually unaltered from 1986-1998. Between 1994-1996 a live diagnostic test for *M. bovis* in badgers was trialled; this was named the 'live test trial' (Bourne *et al.*, 2007). However the trial was abandoned in 1996 due to

concerns over monitoring repeat and contiguous breakdowns without overlap (Krebs *et al.*, 1997).

A further review of the problem of badgers and bovine TB was undertaken by John Krebs (Krebs *et al.*, 1997). The authors of this report, as in Dunnet *et al.* (1986), accepted that badgers were a major cause of *M. bovis* infection in cattle, however they expressed the need for more scientific evidence in order to quantify the effect of badger culling on the control of cattle TB (Krebs *et al.*, 1997). One of the central outcomes of the report was the call for a large-scale scientific investigation to assess the effects of badger culling on the incidence of cattle TB. The report also noted that up until that point in time no badger culling strategy had been rigorously compared to a control strategy of no badger culling.

The Randomised Badger Culling Trial

The Randomised Badger Culling Trial (RBCT) was undertaken as a result of the recommendations made in the report by John Krebs (Krebs *et al.*, 1997), and was overseen by the Independent Scientific Group on Cattle TB (ISG). This replaced the 'interim' strategy of badger culling with a trial designed to test the effects of two different badger culling treatments against each other, and also against a control group of no culling, on the incidence of bovine TB. The two active badger culling strategies were known as 'proactive' and 'reactive' culling. Proactive culling involved culling as many badgers as possible within the designated area, and following this up with subsequent culls (approximately annually) in order to maintain any badger population suppression. Reactive culling involved performing a badger cull only if there was a confirmed herd breakdown, and furthermore, targeting badgers from social groups which had access to the breakdown farm. The trial ran from 1998-2007, and took place across 30 trial areas located in TB 'hotspots' (a 'hotspot' area was broadly defined as an area with a high incidence of bovine TB in cattle) in West and South West England, each area an approximate 100km^2 circular region (see Figure 2.1). These 30 areas were divided into 10 sets of 'triplets', such that areas in the same triplet were relatively close to each other. Within each triplet, one area would be randomly allocated to proactive culling, one to reactive culling, and one to no culling (also known as 'survey-only' areas). One potential problem of assigning different culling treatments to neighbouring areas was that badger territories might have overlapped across area boundaries, and hence invalidating the premise that each area was subjected to just one method of culling. One way to avoid this was to place areas further away from each other, however a key criterion of any trial is to have

Figure 2.1: Map of the ten RBCT triplets, with areas subjected to proactive culling shaded, those areas subjected to reactive culling striped, and survey-only areas empty (Donnelly *et al.*, 2006).

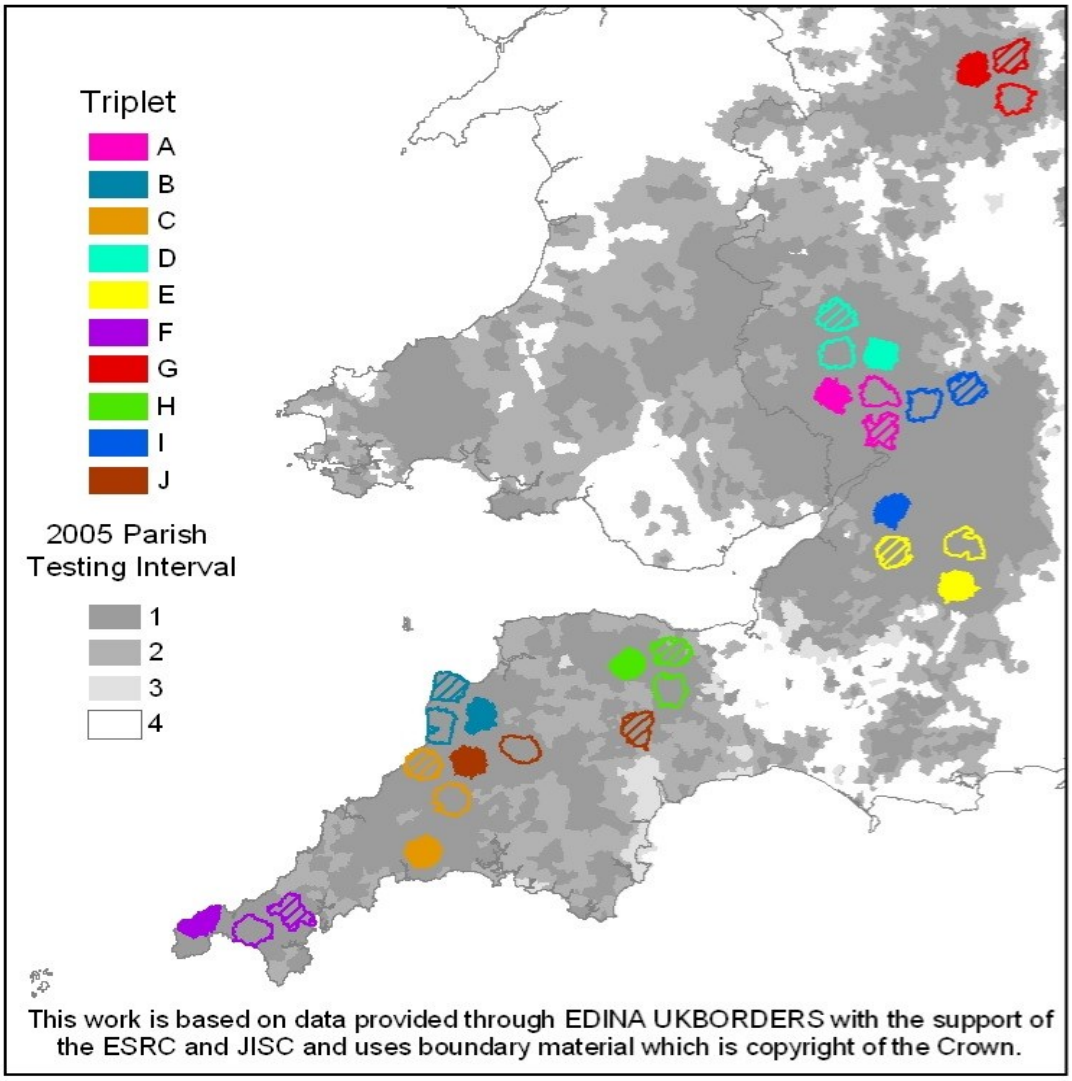
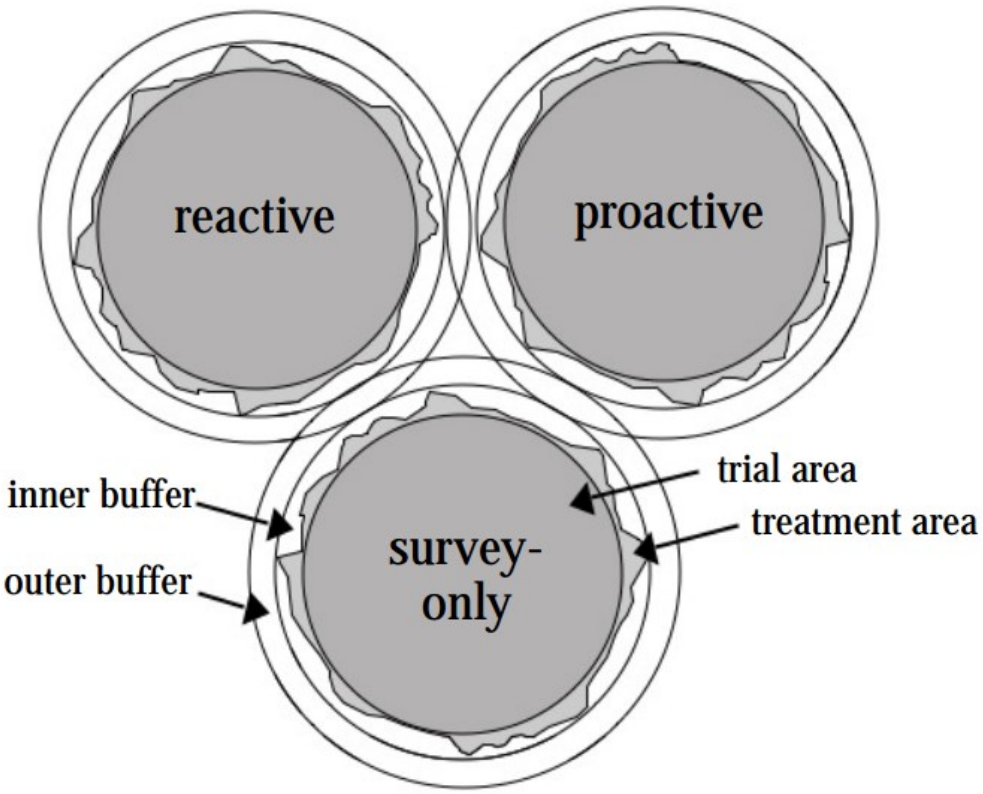


Figure 2.2: Simplified representation of a triplet consisting of the three culling protocols during the RBCT (Bourne *et al.*, 2007).



areas in the same triplet close to each other in order to fairly compare the different treatments. A trade-off was therefore established in the form of having two 'buffer zones' around each area: the first was an 'inner buffer zone', which was 1km in width, and was thought to be the maximum distance most badgers from within that area may travel outside that area. The second was an 'outer buffer zone', which could overlap with neighbouring areas' outer buffer zones to give a 1km distance between the possible ranges of badgers from adjacent groups (Figure 2.2). The RBCT was postponed in 2001 due to the Foot and Mouth Disease outbreak, and resumed in 2002 (Bourne *et al.*, 2007).

The results of the RBCT showed that within the proactive areas of culling the incidence of cattle TB decreased on average by 23.2% (95% confidence interval [CI]: 12.4-32.7%), however in the 2km ring surrounding the culling area there was a 24.5% (95% CI: 0.6% decrease-56.0%) average increase in cattle TB (Donnelly *et al.*, 2007). Early analyses of trial areas subjected to reactive culling showed the incidence of confirmed cattle herd breakdowns had increased by an estimated 27% (95% CI: 2.4% decrease to 65% increase) relative to the control areas, and so as there was a clear and consistent harmful effect on cattle TB levels, this treatment was suspended by Ministers in November 2003 (Donnelly *et al.*, 2003). The observed increases in cattle TB incidence were thought to have been caused by a culling-induced change in badger behaviour known as 'perturbation', where badgers range more widely due to local badger population suppression (MacDonald *et al.*, 2006). Some of these badgers will be infected with *M. bovis*, and so the infection spreads more widely, and consequently to more cattle herds (Jenkins *et al.*, 2007). Perturbation has also been hypothesised to occur in other ecological systems (Abrams, 2009).

History of bovine TB in the Republic of Ireland

The Republic of Ireland has a similar bovine TB problem to the UK. In 1954 there was a national bovine TB elimination programme (Griffin *et al.*, 2005), which included cattle testing, movement restrictions and infection controls. This programme seemed to be effective, and by 1965 the disease prevalence had fallen from 17% in 1954 to 0.5% (O Mairtin *et al.*, 1998b), however this decreasing pattern did not continue. Over time, evidence of a link between badgers, cattle and bovine TB in Ireland too began to mount (Dolan, 1993, Martin *et al.*, 1997, O'Connor and O'Malley, 1989), and since the early 1990s the culling of badgers has been undertaken (Olea-Popelka *et al.*, 2009). There have also been two large-scale field trials in Ireland: "East Offaly Badger Research Project" and "Four-Areas Trial".

The East Offaly Project ran from 1989 to 1995, and badgers within a 528km^2 area were proactively removed under license, with a surrounding 210km^2 'buffer zone', and a further 1456km^2 'control area' around that (O Mairtin *et al.*, 1998a). The Four Areas Trial began in November 1996, with the removal of badgers (again proactively) beginning in September 1997; the trial ended in August 2002, and took place in the counties of Cork North, Donegal, Kilkenny and Monaghan (Griffin *et al.*, 2005). The culls here were undertaken by placing snares for 11-night periods. The data from the Four Areas Trial were also analysed to investigate any spatial or temporal variation in badger numbers (Olea-Popelka *et al.*, 2003). Analyses of both the East Offaly trial and Four Areas Trial concluded that virtual elimination of badgers across large area and maintained over time is likely to have a beneficial effect on cattle TB.

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Chapter 3: Estimating the proportion of badgers (*Meles meles*) culled during the RBCT proactive culls

Abstract

Many disease control programmes designed to tackle the problem of bovine TB in England have included culling badgers (*Meles meles*). Most recently two pilot culls, undertaken by farmers and landowners in areas badly affected by bovine TB, took place in England in late 2013. One of the conditions for obtaining a licence to cull was being able to achieve a badger culling efficacy of at least 70% in the pilot areas. This figure was informed by an indirect estimate of the badger population reduction during the proactive culls of the Randomised Badger Culling Trial (RBCT). In this chapter, genetic and phenotypic data on 8,064 badgers taken during the RBCT were used to assign parents to badger cubs. Direct estimates of the proportion of parents captured during the initial and subsequent culls of the RBCT were generated. A mean of 72.8% (95% bootstrap confidence interval [CI]: 65.6-79.8%) of parents were estimated to have been culled during the initial culls, with no significant difference between the maternal and paternal estimates. A mean of 57.8% (CI: 51.6-64.0%) of mothers and 46.4% (CI: 40.3-52.4%) of fathers were culled during follow-up culls, with the maternal estimate significantly higher than the paternal estimate for follow-up culls ($p = 0.013$).

These results give the first genetically-based estimates of the proportion of adults culled during the initial culls and each of the follow-up culls of the RBCT. The estimate for the proportion of adults captured during the initial culls of 72.8% is in agreement with the 70% culling efficacy requirement for licensed badger culls in England. The corresponding estimates for the follow-up proactive culls can be used to evaluate current or future badger culling-based disease management programmes.

Introduction

Ever since badgers (*Meles meles*) were first associated with bovine TB in 1971 (Muirhead and Gallagher, 1974) there has been stakeholder pressure to reduce badger population sizes in order to control and reduce cattle TB levels in England. Over the last four decades there have been numerous attempts to tackle bovine TB through various culling methods applied to badger populations on different scales. One of the most important quantities estimated from these badger population reduction interventions is the badger removal efficacy of the culling strategy.

The first badger culling operations officially conducted in England used cage-traps to capture badgers in South Dorset in September 1974, however due to poor culling efficacy (Thompson, 1978) this method was replaced in August 1975 with the method of gassing of badger setts. Hydrogen cyanide was used to gas badger setts between 1975-1982, and was assumed to kill badgers relatively quickly in order to minimise suffering (Dunnet *et al.*, 1986). However, experiments conducted in 1982 showed that the dose necessary to achieve a swift, humane death was higher than expected, and so the gassing control programme was halted in June 1982 (Dunnet *et al.*, 1986). The culling methods of the following three decades were all trap-based variants: the clean ring strategy (1982-1986), the interim strategy (1986-1998) and the Randomised Badger Culling Trial (1998-2007). Estimates of culling efficacy in the clean ring and interim strategies were not accurately recorded. In 2011, two pilot badger culls allowing badgers to be culled without the use of traps were authorised, and took place in 2013.

The RBCT was the first large-scale scientific investigation into the effects of badger culling on cattle TB incidence in the UK, with each of the ten sets of trial areas (known as "triplets", with each triplet being assigned a letter A-J) having one area of "proactive" culling, one area of "reactive" culling, and one control area in which no culling took place. Woodroffe *et al.* (2008) derived estimates of the impact of RBCT proactive culling on badger populations by comparing various measures of badger activity in culled and unculled areas. They reported reductions in the density of badger latrines (73%), the density of active badger setts (69%) and the density of road-killed badgers (73%); these figures were summarised as attaining a population reduction of approximately 70% for the RBCT proactive culls (Bourne *et al.*, 2007). Smith and Cheeseman (2007) derived separate estimates of the proportions of badgers captured on initial RBCT proactive culls: for seven of the ten RBCT triplets they estimated a

culling efficacy of 64-77%, whilst for the remaining three triplets they estimated a culling efficacy of 32-39%. Smith and Cheeseman (2007) found a mean culling efficacy over the ten initial proactive culls of 59.9%.

One of the major findings of the RBCT was the substantial effect that badger culling had on the behaviour of badgers, in which surviving badgers in culled areas were more likely to increase their ranging (termed perturbation, Woodroffe *et al.*, 2006). However, there may exist a threshold of culling efficacy below which this social perturbation effect may be of less significance. Given that this effect was clearly present in the follow-up culls of the RBCT (Woodroffe *et al.*, 2006), obtaining an accurate estimate of the culling efficacy during these follow-up culls would also give an upper bound to this threshold value.

More recently, proposals to licence the free shooting of badgers as a method for badger population control were trialled in two pilot areas in West Gloucestershire and West Somerset in late 2013. These culls took place over a six-week period, and allowed farmers and landowners in areas badly affected by bovine TB to shoot free ranging badgers during this period, conditional on obtaining a licence from Natural England. There was much controversy surrounding these culls, particularly in terms of safety, efficacy and humaneness. Consequently, the criteria for being able to obtain a licence to shoot badgers within the two pilot areas was heavily scrutinised, one aspect of which relates to being able to demonstrate that the licence applicant can achieve a culling efficacy of at least 70% (based on the estimates from the RBCT). Results from the two pilot culls of 2013 estimated overall culling efficacy at 27.5-39.1% (95% confidence interval [CI]) in West Gloucestershire (of which 76.7% of badgers culled were killed by free shooting, and 23.3% by cage-traps), and 34.5-48.1% (95% CI) in West Somerset (of which 46.0% of culled badgers were killed by free shooting, and 54.0% by cage-traps). This fell significantly short of the required ≥70% policy requirement, and consequently this method of culling was not extended to more areas of England in 2014, as originally planned if the pilot culls had reached the required targets. However, the government decided to continue culling in the pilot areas for 2014-2016.

Obtaining an accurate estimate of the culling efficacy from the RBCT is therefore of high value to policy decisions, and an improvement in the accuracy of this estimate could be highly valuable. The work presented in this chapter attempts to estimate the culling efficacy through performing parentage analyses using genetic data available from badgers culled during RBCT proactive culls.

Methods

Data

Data used for this work were taken from the 51 proactive culls of the RBCT, which took place over ten areas of land each approximately 100km^2. Badgers were captured using cage-traps and subsequently shot; independent audit found this method to be humane (Anderson, 2005, Anderson, 2006, Ewbank, 2003, Ewbank, 2004, Kirkwood, 2000). Each badger culled had its tooth wear (as an index of age), sex, capture location (to within 100m), length and weight were recorded. Each badger's genetic profile was also obtained (using samples taken from ear tips), consisting of 16 microsatellite loci (a more detailed description of this genetic profiling method can be found in Pope *et al.*, 2007). Hardy-Weinberg and linkage equilibria have been demonstrated for these loci (Carpenter *et al.*, 2005, Pope *et al.*, 2006, Dugdale *et al.*, 2007).

Complete data were available for 8,064 badgers (including 1,611 cubs) taken from 50 of the 51 proactive RBCT culls for this work (there were insufficient data available for the initial proactive cull of triplet A). However, there were also insufficient data available for any of the cubs taken during culls B-2, C-1 and F-3 (using the notation "triplet"-"cull number"). In three triplets there were instances of a pair of same-sex adults with identical microsatellite data (a pair of adult males each had just one loci entry in triplet B, a pair of adult females had 16 loci entries in triplet D, and a pair of adult males had 16 loci entries in triplet F). No cubs had matching microsatellite data.

Genotype error rates were estimated from a wider dataset of 12,119 badgers using Pedant 1.0 (Johnson and Haydon, 2007) on 389 duplicate entries (3.2% of the dataset). This employed a likelihood-based approach to estimate both the allelic dropout rate (0.0063 ± 0.0041 standard deviation [SD]) and the false allele rate (0.0013 ± 0.0014 SD); further details on these error rates can be found in Wang (2004). In order to ensure conservative error rates (as in Sardell *et al.*, 2010) the above rates were inflated to 0.01 and 0.002 respectively. The number of alleles per locus varied between 2 and 12, with a mean value of 6.56.

Data on social group allocation from Woodroffe *et al.* (2009) was also used in the analyses, however this was only available for badgers taken during the initial culls (as reliable social group constructions could not be generated for follow-up culls due to the effects of social perturbation caused by earlier culls, Woodroffe *et al.*, 2009). There were 2,673 badgers in the

above dataset that also had social group allocation information. Badgers were either allocated a unique social group, or were partially (and equally) allocated to between 2 and 4 groups (Woodroffe *et al.*, 2009).

Parentage analyses

Each cub was given a set of possible candidate parents based on the phenotypic data of adults from the same cull as the cub. Each candidate parent was assigned a probability of parentage using a Markov chain Monte Carlo (MCMC) algorithm from the R 2.15.2 package MasterBayes 2.50 (Hadfield *et al.*, 2006), defined as the maternal/paternal assignment probability (MAP/PAP). For both maternity and paternity, the probability that the real parent had not been sampled, defined here as the maternal/paternal null assignment probability (NAP), was also estimated. The total of all MAP values (over all candidate mothers) and the maternal NAP for each cub summed to one, as did the total of all PAP values (over all candidate fathers) and the paternal NAP for each cub.

Parentage assignment methodology

Based on each cub's genetic data, MasterBayes reconstructs every possible pedigree (that is, the genetic make-up of theoretical pairs of candidate parents) based on all alleles present in the genetic data of the set of candidate parents. The program then calculates the relative likelihood of a given pedigree based on the allele frequencies in the genetic data of the pool of candidate parents. This likelihood value is then multiplied by the product of the probability that the parent genotypes would result in the offspring genotype, and an exponential weighting function based on the candidate father's distance from the cub.

That is, for a given cub and a given pedigree p, the relative probability that p is assigned parentage is described by the following proportionality relationship:

$$P(p\ assigned\ parentage) \propto LGe^{-D\lambda}$$

where L is the likelihood of the pedigree based on the allele frequencies in the genetic data;

G is the probability of observing the cub's genetic data given the parent genetic data;

D is the Euclidean distance between the candidate father and the cub;

λ is a model parameter relating to father-cub distance.

Specifically, the value of G is based on the Mendelian probability of obtaining the observed alleles of the cub based on the observed alleles of both parents, for all 16 loci (Thompson, 1976). If, for a given locus, a candidate mother has alleles a_m and b_m, and a candidate father has alleles a_f and b_f, then the allelic probability distribution of an offspring cub of these candidate parents at this locus would take the following form:

Cub allele combination		**Probability of allele combination**
cub allele 1	*cub allele 2*	
a_m	b_m	0.25
a_m	b_f	0.25
a_f	b_m	0.25
a_f	b_f	0.25
any other allele combination		0

For reconstructed pedigrees in which the candidate father was not captured, values for D are randomly drawn from the same distribution of values for D from observed candidate fathers (Hadfield *et al.*, 2006). The resultant values for all pedigrees consisting of a candidate mother/father that was unsampled are assigned into the "unsampled" mother/father category.

Unlike many alternative parentage analysis programs that estimate parentage and other model parameters separately (Marshall *et al.*, 1998), MasterBayes employs a full-probability model, meaning that both parentage and other model parameters (λ) are estimated simultaneously. Whilst this means that MasterBayes is designed to produce the "exact" answer, it also means that it is substantially more computationally intensive to systematically find the optimal set of parameters. Therefore MasterBayes uses an MCMC algorithm to estimate parameter values; details of this MCMC parameter estimation algorithm can be found in Hadfield *et al.* (2006).

Candidate parents

It was computationally unfeasible to test every possible mother-father pairing from each cull due to the large number of combinations, and so the following two methods of parentage analyses were performed:

"all culls" method

In this method, the set of candidate mothers for each cub was defined to be all adult females who were captured:

1. from the same trial area as the cub;
2. within 1km of the cub;
3. during the same cull as the cub.

A cut-off distance of 1km was chosen to be a realistic upper bound on the distance between a mother and a cub for candidate mothers taken during initial culls, as field studies in agricultural areas have shown badger territory sizes and adult female home ranges to be much smaller than the equivalent area of a circle of radius 1km (Cheeseman *et al.*, 1981, Palphramand *et al.*, 2007). During the follow-up culls, badger home ranges were known to increase due to perturbation caused by prior culls (Pope *et al.*, 2007, Woodroffe *et al.*, 2006). However, this effect was found to be significantly greater in males than females (Pope *et al.*, 2007). Moreover, field studies analysing the change in extra-group movement following a badger removal operation found that all adult females who re-colonised completely or partially culled groups showed no signs of ever having lactated (Tuyttens *et al.*, 2000), and no adult females that were known to have lactated previously were recorded to have re-colonised such groups. The same mother-cub distance cut-off value of 1km was therefore deemed plausible for candidate mothers from follow-up culls too.

The set of candidate fathers was defined to be all adult males who were captured:

1. from the same trial area as the cub;
2. during the same cull as the cub.

No distance-defined restriction was applied to candidate fathers, as adult males are thought to move between groups more than females (Macdonald *et al.*, 2008) meaning fathers may be members of a different social group to the cub (Dugdale *et al.*, 2007). Therefore fathers are likely to be captured at greater distances from the cub than mothers. However, an exponential probabilistic weighting function was applied such that for a given genetic profile, candidate fathers captured closer to the cub had a greater PAP, as earlier genetic parentage work also shows that just over half of fathers reside in the same social group as their cubs (Dugdale *et al.*, 2007).

The above “all culls” method of choosing candidate parents was used as the primary parentage analysis for this work, as this gives a direct estimate of the proportion of parents culled during the same cull as the cub (by summing the MAPs and PAPs) for all RBCT proactive culls, and thus an estimate of the proportion of adults culled during each cull.

Table 3.1: Tuning parameters and iteration count for models used in the (a) "all culls" analyses, (b) "initial culls" analyses. Culls listed in the same row indicate that cubs from these culls were analysed using the same model. ** The 156 cubs from cull E-1 were split into three cohorts of 52 for model runs.

(a)

RBCT cull(s)	Tuning parameters			Number of iterations
	USdam	USsire	β	
A-2	0.22	0.22	7	10,000,000
A-3, A-4, A-5, B-1, B-3, B-4 & B-7	0.18	0.18	11	20,000,000
B-5	0.22	0.22	20	10,000,000
B-6	0.22	0.22	0.02	10,000,000
C-2, C-3, C-5 & C-6	0.05	0.05	3	10,000,000
C-4	0.5	0.5	2.5	20,000,000
D-1	0.4	0.4	10	10,000,000
D-2	0.125	0.125	10	10,000,000
D-3	0.3	0.3	50	10,000,000
D-4, E-2 & E-3	0.15	0.15	5	20,000,000
E-1a*	0.3	0.3	22	20,000,000
E-1b*	0.2	0.2	22	10,000,000
E-1c*	0.2	0.2	120	10,000,000
E-4	0.05	0.05	5	10,000,000
E-5	0.18	0.18	10	20,000,000
E-6	0.22	0.22	40	10,000,000
F-1	0.07	0.07	25	10,000,000
F-2	0.3	0.3	23	10,000,000
F-4	0.6	0.6	50	20,000,000
F-5	0.25	0.25	200	10,000,000
G-1	0.15	0.15	10	20,000,000
G-2	0.115	0.115	1.5	10,000,000
G-3	0.15	0.15	1.5	10,000,000
G-4	0.2	0.2	3.5	10,000,000
G-5	0.1	0.1	20	10,000,000
H-1, H-3, H-4 & H-5	0.15	0.15	10	10,000,000
H-2	0.1	0.1	22	20,000,000
I-1	0.08	0.08	8	10,000,000
I-2	0.15	0.15	12	20,000,000
I-3, J-3 & J-4	2	2	85	10,000,000
I-4	0.05	0.05	0.4	10,000,000
J-1	0.25	0.25	45	10,000,000
J-2	0.05	0.05	0.5	10,000,000

(b)

RBCT cull(s)	Tuning parameters			Number of iterations
	USdam	USsire	β	
B-1, H-1 & I-1	0.15	0.15	15	15,000,000
D-1	0.5	0.5	10	15,000,000
E-1a**	0.35	0.35	33	15,000,000
E-1b**	0.25	0.25	30	5,000,000
E-1c**	0.12	0.12	90	5,000,000
F-1	0.1	0.1	22	5,000,000
G-1	0.1	0.1	4	15,000,000
J-1	0.175	0.175	45	15,000,000

A second method of choosing candidate parents based on badger social group assignments (for cubs captured during the initial culls only) was also performed:

"initial culls" method

This method was based on badger social group constructions, as described in Woodroffe *et al.* (2009), generated for badgers captured during the initial culls of the RBCT. The set of candidate mothers for each cub under this method was defined to be adult females who could have been from the same social group (and thus the same cull) as the cub, based on either unique or partial social group allocations. The set of candidate fathers was defined to be adult males who were captured during the same cull as the cub, and the same PAP-weighting condition was used as under the "all culls" method (applied only to initial culls in this method).

Other values estimated during the parentage analyses were the number of adult females that were not sampled for each cub (denoted 'USdam') and the number of adult males not sampled for each cub (denoted 'USsire'). All tuning parameters were chosen so that the Metropolis-Hastings acceptance rates for the MCMC chains were between 0.2 and 0.5, and these values for each model run can be found in Table 3.1. A burn-in value of 250,000 and a thinning interval of 5,000 were used in all model runs, and the number of iterations for each model can also be found in Table 3.1. Autocorrelation values of all parameters in all model runs were below 0.05.

For both the "all culls" and "initial culls" methods, the set of candidate mothers for each cub was different, and so the resultant USdam parameter estimate was of no use in estimating the number of adult females not culled. For culls in which there were too few or too many cubs for the parentage analyses to be performed for just that cull, analyses for several culls were combined (in cases of too few cubs) or split into several runs (in cases of too many cubs); the resultant parameter estimates of USsire from these runs were therefore not of use in estimating the number of adult males not captured during a specific cull. In cases where model runs did not include combined or split culls, the resultant USsire parameter value could be used to estimate the overall number of adult males not sampled for that cull, and thus by using the number of sampled adult males, could be used to estimate to the proportion of adult males not culled.

Statistical analyses

For each cub, the sum of all MAPs over all candidate mothers under the "all culls" method made up the probability that the mother was culled during the same cull as the cub (or equivalently, one minus the maternal NAP), and the sum of all PAPs over all candidate fathers under the "all culls" method made up the probability that the father was culled during the same cull as the cub (or, one minus the paternal NAP). The mean value for the probability that a cub's mother/father was culled during the same cull as the cub was calculated for each cull. The mean of these mother and father same-cull capture probabilities gave the mean probability a parent was culled for each cull. Equivalent mother and father same-cull capture probabilities were also calculated using parentage results under the "initial culls" method.

For the "all culls" method, confidence intervals for the mean values over the initial culls and each set of follow-up culls were generated using 10,000 bootstraps with replacement over the ten RBCT triplets. For each bootstrap, ten triplets were chosen with replacement and the mean probability that a mother/father was culled for each cull over the initial culls, and over each set of follow-up culls, was recorded. The values 2.5th and 97.5th percentiles gave the lower and upper confidence bounds, respectively. Confidence intervals for estimates over all follow-up culls combined were also constructed using 10,000 bootstraps with replacement over all the 41 follow-up RBCT culls, with the 2.5th and 97.5th percentiles of the resultant means used as the lower and upper confidence bounds, respectively. For the "initial culls" method, confidence intervals for mean values across all initial culls were generated using bootstraps taken from the eight triplets (with replacement) in which sufficient data was available (so excluding triplets A and C). Bootstrap confidence intervals were also generated for the estimate of the mean proportion of badgers culled during the initial culls of the RBCT from Smith and Cheeseman (2007). To test for differences between means, p-values were calculated from the 10,000 bootstrap samples associated with each mean value.

Table 3.2: Estimates of the percentage of (a) mothers, (b) fathers, and (c) parents culled and genotyped for each cull of the RBCT under the "all culls" method of parentage analysis, by triplet, with 95% bootstrap confidence intervals for means over the initial culls and each set of follow-up culls.

(a)

RBCT triplet	Initial cull	Second cull	Third cull	Fourth cull	Fifth cull	Sixth cull	Seventh cull
A		84.9	82.6	86.7	40.8		
B	63.7		22.2	44.4	65.5	48.4	83.5
C		63.3	59.4	88.2	47.5	64.4	
D	51.2	83.5	55.3	79.9			
E	89.9	37.4	39.2	43.0	26.9	38.3	
F	82.5	94.7		84.6	68.3		
G	78.9	60.4	42.7	51.2	44.2		
H	82.3	63.6	32.1	37.8	24.8		
I	52.7	75.3	68.5	31.9			
J	82.7	64.7	46.4	77.3			
Total	**73.0** (63.0, 82.5)	**69.8** (58.8, 80.0)	**49.8** (38.5, 61.4)	**62.5** (49.2, 75.6)	**45.4** (33.7, 57.3)	**50.4** (38.3, 64.4)	**83.5** (not available)

(b)

RBCT triplet	Initial cull	Second cull	Third cull	Fourth cull	Fifth cull	Sixth cull	Seventh cull
A		16.7	47.7	8.2	46.4		
B	66.0		50.4	83.1	59.0	26.1	8.8
C		93.4	40.6	56.7	35.2	30.5	
D	86.2	47.9	51.0	42.6			
E	80.2	61.0	42.7	67.3	62.7	56.4	
F	85.4	82.4		72.1	49.5		
G	86.2	46.1	57.8	29.1	47.4		
H	48.8	56.7	23.6	12.2	37.9		
I	59.8	40.3	61.6	39.7			
J	68.5	61.0	25.1	32.4			
Total	**72.6** (63.0, 81.3)	**56.2** (42.2, 69.9)	**44.5** (36.2, 52.3)	**44.3** (29.8, 59.4)	**48.3** (41.3, 55.4)	**37.7** (26.1, 56.4)	**8.8** (not available)

(c)

RBCT triplet	Initial cull	Second cull	Third cull	Fourth cull	Fifth cull	Sixth cull	Seventh cull
A		50.8	65.2	47.5	43.6		
B	64.9		36.3	63.8	62.3	37.3	46.2
C		78.4	50.0	72.5	41.4	47.5	
D	68.7	65.7	53.2	61.3			
E	85.1	49.2	41.0	55.2	44.8	47.4	
F	84.0	88.6		78.4	58.9		
G	82.6	53.3	50.3	40.2	45.8		
H	65.6	60.2	27.9	25.0	31.4		
I	56.3	57.8	65.1	35.8			
J	75.6	62.9	35.8	54.9			
Total	**72.8** (65.6, 79.8)	**63.0** (55.3, 71.8)	**47.2** (39.3, 55.2)	**53.4** (43.5, 63.1)	**46.9** (39.6, 54.4)	**44.0** (37.3, 47.5)	**46.2** (not available)

Table 3.3: β and USsire parameter estimates for each model analysed as a whole unique cull, together with the number of sampled adult males from these culls and the resultant estimates of the proportion of adult males culled (based on the USsire estimates) using (a) the "all culls" data, (b) the "initial culls" data.

(a)

RBCT cull	β	USsire	Number of adult males sampled	Percentage culled (%)
A-2	-0.0014	171.7	24	12.3
B-5	-0.0022	46.1	56	54.8
B-6	-0.0025	138.3	24	14.8
C-4	-0.0012	50.6	41	44.8
D-1	-0.0038	48.8	152	75.7
D-2	-0.0017	108.3	92	45.9
D-3	-0.0029	87	68	43.9
E-4	-0.0015	35.3	67	65.5
E-5	-0.0018	41.4	55	57.1
E-6	-0.0027	70.3	70	49.9
F-1	-0.0026	29.2	155	84.1
F-2	-0.0019	21.6	83	79.3
F-4	-0.0025	52.9	84	61.4
F-5	-0.0060	74	53	41.7
G-1	-0.0018	52.5	162	75.5
G-2	-0.0025	76.4	58	43.2
G-3	-0.0018	44.9	50	52.7
G-4	-0.0015	122.6	33	21.2
G-5	-0.0020	41.7	31	42.6
H-2	-0.0030	47.4	57	54.6
I-1	-0.0018	57.3	73	56.0
I-2	-0.0020	82.9	45	35.2
I-4	-0.0021	116	33	22.1
J-1	-0.0031	81.8	153	65.2
J-2	-0.0014	37.3	51	57.8

(b)

RBCT cull	β	USsire	Number of adult males sampled	Percentage culled
D-1	-0.0033	45.6	152	76.9%
F-1	-0.0023	26.2	155	85.5%
G-1	-0.0017	70.1	162	69.8%
J-1	-0.0033	58.8	153	72.2%

Table 3.4: The total number of (a) badgers captured during the proactive culls of the RBCT by triplet, (b) adults with known infection status, and (c) cubs with known infection status (35 badgers that had unknown age, gender or infection status were included in (a) but not in (b) or (c)). † The total for both the initial cull and first follow-up culls in triplet E.

(a)

RBCT triplet	1998	1999	2000	2002	2003	2004	2005	Total
A		55		149	52	58	48	**362**
B	238	85	74	49	172	111	58	**787**
C		246	111	126	132	187	162	**964**
D				293	369	211	181	**1054**
E			744†	96	258	214	148	**1460**
F			451	249	103	220	155	**1178**
G			427	205	144	103	117	**996**
H			162	231	71	74	53	**591**
I				219	176	93	172	**660**
J				441	187	109	109	**846**
Total	**238**	**386**	**1969**	**2058**	**1664**	**1380**	**1203**	**8898**

(b)

RBCT triplet	1998	1999	2000	2002	2003	2004	2005	Total
A		55		86	46	42	46	**275**
B	229	82	66	43	125	72	55	**672**
C		236	107	85	114	121	148	**811**
D				277	274	183	159	**893**
E			563†	78	165	148	119	**1073**
F			337	200	103	202	125	**967**
G			409	131	108	70	75	**793**
H			145	137	57	60	44	**443**
I				169	129	89	86	**473**
J				396	128	107	98	**729**
Total	**229**	**373**	**1627**	**1602**	**1249**	**1094**	**955**	**7129**

(c)

RBCT triplet	1998	1999	2000	2002	2003	2004	2005	Total
A		0		60	6	16	2	**84**
B	9	0	8	6	45	39	1	**108**
C		7	3	41	18	66	14	**149**
D				15	95	27	22	**159**
E			180†	17	91	66	29	**383**
F			109	49	0	18	30	**206**
G			15	72	36	33	42	**198**
H			16	94	14	14	9	**147**
I				49	47	4	84	**184**
J				46	57	2	11	**116**
Total	**9**	**7**	**331**	**449**	**409**	**285**	**244**	**1734**

Table 3.5: The total number of (a) adults and (b) cubs culled during the RBCT proactive culls with known age, gender, infection status and with sufficient genetic data (% of (a) adults and (b) cubs with known age, gender and infection status and with sufficient genetic data).

(a)

RBCT triplet	Initial cull	Second cull	Third cull	Fourth cull	Fifth cull	Sixth cull	Seventh cull	Total
A	0 (0.0%)	82 (95.3%)	45 (97.8%)	42 (100.0%)	22 (47.8%)			**191** (69.5%)
B	147 (64.2%)	61 (74.4%)	65 (98.5%)	43 (100.0%)	122 (97.6%)	49 (68.1%)	53 (96.4%)	**540** (80.4%)
C	2 (0.8%)	103 (96.3%)	85 (100.0%)	113 (99.1%)	79 (65.3%)	144 (97.3%)		**526** (64.9%)
D	276 (99.6%)	266 (97.1%)	179 (97.8%)	156 (98.1%)				**877** (98.2%)
E	429 (97.7%)	117 (94.4%)	76 (97.4%)	163 (98.8%)	104 (70.3%)	117 (98.3%)		**1006** (93.8%)
F	323 (95.8%)	197 (98.5%)	101 (98.1%)	200 (99.0%)	125 (100.0%)			**946** (97.8%)
G	389 (95.1%)	125 (95.4%)	105 (97.2%)	70 (100.0%)	74 (98.7%)			**763** (96.2%)
H	142 (97.9%)	135 (98.5%)	57 (100.0%)	38 (63.3%)	43 (97.7%)			**415** (93.7%)
I	166 (98.2%)	128 (99.2%)	88 (98.9%)	82 (95.3%)				**464** (98.1%)
J	396 (100.0%)	125 (97.7%)	106 (99.1%)	98 (100.0%)				**725** (99.5%)
Total	**2270** (84.3%)	**1339** (95.8%)	**907** (98.4%)	**1005** (96.7%)	**569** (83.2%)	**310** (91.4%)	**53** (96.4%)	**6453** (90.5%)

(b)

RBCT triplet	Initial cull	Second cull	Third cull	Fourth cull	Fifth cull	Sixth cull	Seventh cull	Total
A		57 (95%)	6 (100.0%)	16 (100.0%)	2 (100.0%)			**81** (96.4%)
B	7 (77.8%)		8 (100.0%)	6 (100.0%)	43 (95.6%)	26 (66.7%)	1 (100.0%)	**91** (84.3%)
C		3 (100.0%)	41 (100.0%)	18 (100.0%)	42 (63.6%)	14 (100.0%)		**118** (79.2%)
D	15 (100.0%)	94 (98.9%)	27 (100.0%)	22 (100.0%)				**158** (99.4%)
E	156 (96.3%)	6 (33.3%)	16 (94.1%)	89 (97.8%)	34 (51.5%)	28 (96.6%)		**339** (88.5%)
F	105 (96.3%)	49 (100.0%)		18 (100.0%)	30 (100.0%)			**202** (98.1%)
G	15 (100.0%)	64 (88.9%)	35 (97.2%)	33 (100.0%)	41 (97.6%)			**188** (94.9%)
H	13 (81.3%)	93 (98.9%)	14 (100%)	11 (78.6%)	9 (100.0%)			**140** (95.2%)
I	48 (98%)	46 (97.9%)	4 (100.0%)	83 (98.8%)				**181** (98.4%)
J	46 (100.0%)	54 (94.7%)	2 (100.0%)	11 (100.0%)				**113** (97.4%)
Total	**405** (94.6%)	**476** (96.2%)	**153** (98.7%)	**307** (98.1%)	**201** (77.3%)	**68** (82.9%)	**1** (100.0%)	**1611** (92.9%)

Results

"all culls"

For cubs culled during the initial culls, a mean of 73.0% (95% bootstrap confidence interval [CI]: 63.0-82.5%, Table 3.2(a)) of mothers and 72.6% (CI: 63.0-81.3%, Table 3.2(b)) of fathers were culled and genotyped. Over all follow-up culls, a mean of 57.8% (CI: 51.6-64.0%) of mothers and 46.4% (CI: 40.3-52.4%) of fathers were culled and genotyped. For each set of follow-up culls, the mean percentage of mothers culled and genotyped ranged between 45.4% and 83.5%, and the mean percentage of fathers culled and genotyped ranged between 8.8% and 56.2% (specific mean values and confidence intervals for all maternal and paternal estimates can be found in Tables 3.2(a) and 3.2(b)).

During the initial culls a significantly higher mean percentage of both mothers and fathers were culled and genotyped than over all follow-up culls combined ($p = 0.012$ and $p < 0.001$ respectively). There was no significant difference between the estimates of the percentage of mothers and the percentage of fathers culled and genotyped for the initial culls, however there were significantly more mothers culled and genotyped over all follow-up culls combined than fathers ($p = 0.013$). There was no significant difference between the estimates of the percentage of mothers and fathers culled and genotyped for any one set of follow-up culls alone.

The mean percentage of parents culled and genotyped during the initial culls was 72.8% (CI: 65.6-79.8%, Table 3.2(c)), and for each follow-up cull ranged between 44.0% and 63.0% (specific mean values and confidence intervals can be found in Table 3.2(c)).

For culls in which parentage could be estimated for all cubs within a single model, estimates of the USsire model parameter can be found in Table 3.3. The corresponding estimate of the mean percentage of fathers culled and genotyped over these culls was 50.3%. Estimates of the value of β, describing the exponential paternal distance weighting function, can also be found for each cull in Table 3.3.

Sensitivity analysis

Similar estimates of the percentage of mothers and fathers culled were generated using data just from culls in which over 90% of adults were genotyped. Tables 3.4 and 3.5 show the breakdown of the number of badgers taken during the RBCT by age and information

Table 3.6: Estimates of the percentage of (a) mothers, (b) fathers, and (c) parents culled and genotyped for each cull of the RBCT under the "all culls" method of parentage analysis, by triplet, with 95% bootstrap confidence intervals for means over the initial culls and each set of follow-up culls (excluding culls where fewer than 90% of culled adults were genotyped).

(a)

RBCT triplet	Initial cull	Second cull	Third cull	Fourth cull	Fifth cull	Sixth cull	Seventh cull
A		84.9	82.6	86.7			
B			22.2	44.4	65.5		83.5
C		63.3	59.4	88.2		64.4	
D	51.2	83.5	55.3	79.9			
E	89.9	37.4	39.2	43.0		38.3	
F	82.5	94.7		84.6	68.3		
G	78.9	60.4	42.7	51.2	44.2		
H	82.3	63.6	32.1		24.8		
I	52.7	75.3	68.5	31.9			
J	82.7	64.7	46.4	77.3			
Total	**74.3** (62.6, 84.3)	**69.8** (59.2, 79.9)	**49.8** (38.4, 61.5)	**65.2** (51.2, 78.6)	**50.7** (29.7, 67.6)	**51.4** (38.3, 64.4)	**83.5** (not available)

(b)

RBCT triplet	Initial cull	Second cull	Third cull	Fourth cull	Fifth cull	Sixth cull	Seventh cull
A		16.7	47.7	8.2			
B			50.4	83.1	59.0		8.8
C		93.4	40.6	56.7		30.5	
D	86.2	47.9	51.0	42.6			
E	80.2	61.0	42.7	67.3		56.4	
F	85.4	82.4		72.1	49.5		
G	86.2	46.1	57.8	29.1	47.4		
H	48.8	56.7	23.6		37.9		
I	59.8	40.3	61.6	39.7			
J	68.5	61.0	25.1	32.4			
Total	**73.6** (62.9, 83.6)	**56.2** (42.1, 70.0)	**44.5** (35.9, 52.2)	**47.9** (33.3, 62.2)	**48.5** (40.2, 56.6)	**43.5** (30.5, 56.4)	**8.8** (not available)

(c)

RBCT triplet	Initial cull	Second cull	Third cull	Fourth cull	Fifth cull	Sixth cull	Seventh cull
A		50.8	65.2	47.5			
B			36.3	63.8	62.3		46.2
C		78.4	50.0	72.5		47.5	
D	68.7	65.7	53.2	61.3			
E	85.1	49.2	41.0	55.2		47.4	
F	84.0	88.6		78.4	58.9		
G	82.6	53.3	50.3	40.2	45.8		
H	65.6	60.2	27.9		31.4		
I	56.3	57.8	65.1	35.8			
J	75.6	62.9	35.8	54.9			
Total	**74.0** (65.8, 81.3)	**63.0** (55.5, 71.4)	**47.2** (39.2, 55.4)	**56.6** (47.8, 65.4)	**49.6** (35.0, 61.4)	**47.4** (47.4, 47.5)	**46.2** (not available)

Table 3.7: Estimates of the percentage of mothers, fathers and parents culled and genotyped for each initial cull of the RBCT under the "initial culls" method of parentage analysis, by triplet, with 95% bootstrap confidence intervals.

RBCT triplet	A	B	C	D	E	F	G	H	I	J	Overall
Maternal		61.4		53.8	83.8	81.9	69.5	64.2	53.7	52.6	**65.1** (57.5, 73.3)
Paternal		81.2		86.7	81.6	86.9	81.2	69.5	71.7	75.7	**79.3** (75.0, 83.4)
Parental		71.3		70.3	82.7	84.4	75.4	66.9	62.7	64.2	**72.2** (67.2, 77.7)

Table 3.8: Comparison of the estimates of the percentage of parents culled during the initial culls of the RBCT under the "all culls" and "initial culls" methods against estimates from Smith and Cheeseman (2007), with 95% bootstrap confidence intervals.

RBCT triplet	A	B	C	D	E	F	G	H	I	J	Overall
"all culls"		64.9		68.7	85.1	84	82.6	65.6	56.3	75.6	**72.8** (65.6, 79.8)
"initial culls"		71.3		70.3	82.7	84.4	75.4	66.9	62.7	64.2	**72.2** (67.2, 77.7)
Smith and Cheeseman	32.0	76.6	69.0	37.3	73.1	66.3	68.8	64.2	39.4	72.7	**59.9** (49.5, 68.8)

available; in particular, Table 3.5(a) shows that less than 90% of adults were genotyped from culls A-5, B-1, B-6, C-5, E-5 and H-4 (using the "triplet"-"cull number" notation), and so were excluded from the sensitivity analysis.

For the initial culls, a mean of 74.3% (CI: 62.6-84.3%, Table 3.6(a)) of mothers and 73.6% (CI: 62.9-83.6%, Table 3.6(b)) of fathers were culled and genotyped. Over all follow-up culls, a mean of 60.4% (CI: 53.4-67.1%) of mothers and 47.8% (CI: 41.2-54.5%) of fathers were culled and genotyped. For each set of follow-up culls, the mean percentage of mothers culled and genotyped ranged between 49.8% and 83.5%, and the mean percentage of fathers culled and genotyped ranged between 8.8% and 56.2% (specific mean values and confidence intervals for all maternal and paternal estimates can be found in Tables 3.6(a) and 3.6(b)).

All statistically significant findings in the "all culls" analyses were also found in these sensitivity analyses. The mean percentage of both mothers and fathers culled and genotyped during the initial culls were again significantly higher than over all follow-up culls combined ($p = 0.046$ and $p < 0.001$ respectively), and there were significantly more mothers culled and genotyped over all follow-up culls combined than fathers ($p = 0.011$). As found in the "all culls" analyses, there was again no significant difference between the estimates of the percentage of mothers and the percentage of fathers culled and genotyped for the initial culls or for any one set of follow-up culls. The mean percentage of parents culled and genotyped during the initial culls was 74.0% (CI: 65.8-81.3%, Table 3.6(c)), and for each follow-up cull ranged between 46.2% and 63.0% (specific mean values and confidence intervals can be found in Table 3.6(c)).

"initial culls"

Only estimates of the percentage of cubs culled and genotyped during the initial culls could be generated under this method, for which a mean of 65.1% (CI: 57.5-73.3%, Table 3.7(a)) of mothers and 79.3% (CI: 75.0-83.4%, Table 3.7(b)) of fathers were culled and genotyped. There was a significant difference between the percentage of mother and fathers culled and genotyped ($p = 0.002$). However, the overall mean percentage of parents culled and genotyped based on these figures was 72.2% (CI: 67.2-77.7%, Table 3.7(c)), and thus was very similar to the initial cull estimate of 72.8% under the "all culls" method.

Initial cull comparison

The estimates of the percentage of mothers culled and genotyped obtained for the initial culls using the "all culls" and "initial culls" methods (Table 3.8) were not significantly different; similarly, estimates of the percentage of fathers culled and genotyped were not significantly different between the two methods either. However, the estimates of the percentage of parents culled and genotyped during the initial culls using either the "all culls" or "initial culls" method were significantly higher than those found in Smith and Cheeseman (2007) (Table 3.8, $p = 0.029$ and $p = 0.017$ respectively).

Time series analysis

To assess whether there was a significant trend in the mean values compared with the cull number of the triplet, two time series analyses were performed: one over all culls, and a second over all follow-up culls. When analysing all culls, there was an estimated decrease of 3.9% (95% CI: 0.3-7.4%) in the mean proportion of parents culled over successive cull numbers ($p = 0.037$); the results of the analysis for the follow-up culls showed no significant trend ($p = 0.290$).

Discussion

This work estimates that a mean of 72.8% (CI: 65.6-79.8%) of parents were culled and genotyped during the initial culls of the RBCT, and a mean of 57.8% (CI: 51.6-64.0%) of mothers and 46.4% (CI: 40.3-52.4%) of fathers were culled and genotyped over all follow-up culls of the RBCT based on the "all culls" analyses.

The significantly lower values for both the maternal and paternal means over the follow-up culls compared to the initial culls is likely to be due in part to the presence of more trap-shy individuals during later culls, as trap-happy individuals were more likely to have been culled during the initial culls. Additionally, social perturbation of badgers during the follow-up culls (Pope *et al.*, 2007, Woodroffe *et al.*, 2006) may have meant parents were further away from their cubs, and thus fewer parents were culled or accurately assigned. Furthermore, lower badger densities during the follow-up culls (caused by the removal of badgers during the initial culls) could have also meant that there was less stress on individuals competing for food, and thus badgers were less likely to enter the traps for the bait. Follow-up culls were also known to have an increased proportion of badgers caught close to the culling area boundary compared to earlier culls (Woodroffe *et al.*, 2008), meaning that more badgers might have been outside the culling area during later culls too. Therefore cubs near the culling boundary may have also had a lower probability of parentage assignment. To test this hypothesis, a binary variable of whether or not the cub was caught within 1km of the culling boundary was used as the outcome variable in a logistic regression model against cubs' null MAP and null PAP values (the probability a cub's mother and father, respectively, were not culled); however both were non-significant ($p = 0.10$ and $p = 0.07$ respectively).

The difference between the mean maternal and paternal estimates was significantly different for the follow-up culls combined (57.8% to 46.4% respectively), whereas the difference between the corresponding estimates of the initial culls were non-significant (73.0% to 72.6% respectively). As mentioned above, a possible reason for the lower estimates during the follow-up culls than the initials culls could be due to social perturbation. Noting that male badgers range significantly more widely than female badgers after a cull (Pope *et al.*, 2007), we might expect a more substantial decrease in the paternal estimate of the follow-up culls due to fathers moving away (or at least be further away from their social group at the time of culling) than the decrease in the maternal estimate. The fact that the mean paternal estimate was significantly lower than the mean maternal estimate over the follow-up culls, but was not

significantly different for the initial culls, would indeed suggest that fathers had moved further away than mothers due to culling. However, whilst parents may move away from a certain area due to culling, other badgers from outside may move into this area. Therefore although the parents may have moved away, the local population size may not have substantially changed (after accounting for badgers lost due to culling). Therefore the paternal estimate for the follow-up culls (46.4%) is likely to be more of an underestimate of the true proportion of adult males culled during the follow-up culls than the maternal estimate for the follow-up culls (57.8%) is of the true proportion of adult females culled during the follow-up culls. Furthermore, Tuyttens *et al.* (2000) found that mothers were less likely to move from their social group after a badger removal operation than non-mothers, with no evidence of the same being true for fathers compared to non-fathers, suggesting that the maternal estimate of 57.8% would be a better estimate of the true adult female culling efficacy for the follow-up culls.

Based on the hypothesis that perturbation impacts on the accuracy of using parental culling efficacy as a measure of adult trapping efficacy, the probability that a cub's mother/father was culled was compared to the month the cub was culled. This was to test for any significant changes between monthly parental capture probabilities, which may highlight any months during which mothers/fathers were significantly more likely to be near to the cub (and thus estimates of true adult trapping efficacy during these months would be less affected by perturbation). However, there was no significant association between the probability a cub's mother/father was culled and the month of the cull.

The only results from these analyses that were not affected by perturbation due to previous culling were those from the initial culls, in which maternal and paternal estimates were very similar for the "all culls" results. Therefore it could be the case that true adult female and adult male culling efficacies during the follow-up culls are also of a similar magnitude, and so the estimate of 57.8% for the true adult female culling efficacy during the follow-up culls may also be valid for estimating the true adult male culling efficacy during the follow-up culls, and thus adult trapping efficacy during the follow-up culls in general.

It was not possible, using these methods, to estimate the proportion of cubs that were not culled. However, cubs are known to be more trappable than adults when traps are pre-baited (Tuyttens *et al.*, 1999), as in the RBCT, and so these estimates may provide an approximate lower bound for cub culling efficacies too.

Results from the sensitivity analysis made little difference, with just a slight increase in mean values where culls had been removed from the analyses due to low adult genotyping rates.

The estimate of the mean percentage of parents not culled and genotyped during the initial culls under the "initial culls" method (72.2%, CI: 67.2-77.7%) was very similar to the "all culls" estimate of 72.8% (CI: 65.6-79.8%), and both the maternal and paternal estimates of the "initial culls" method were not significantly different to the corresponding estimates obtained for the initial culls using the "all culls" method. However, the difference between the maternal and paternal estimate under the "initial culls" method was highly significant ($p = 0.003$). This could be due to mothers not being assigned the correct social group, possibly because they were in a neighbouring social group territory when they were captured and culled.

Both of the estimates of the mean proportion of parents culled during the initial culls were significantly higher than the corresponding mean found in Smith and Cheeseman (2007) (excluding triplets A and C). The trap-based culling undertaken in the RBCT would have meant that trap-shy cubs were less likely to have been culled and thus less likely to be present in the data. If parents of such cubs were also trap-shy, then it is possible that the mean capture rates presented here could be overestimates.

As outlined above, one candidate mechanism explaining the lower success rate of parentage assignment during the follow-up culls compared to the initial culls is the effect of social perturbation. This mechanism would also potentially explain the more pronounced decrease in success on successive culls in paternity rather than maternity, on the assumption that perturbation affects adult males more than adult females. If social perturbation is the underlying mechanism driving this difference, then it is likely that parents of some cubs captured during follow-up culls will also have been captured, but not assigned, due to them being too far from the cub. This would suggest that the estimates of the culling efficacies of the follow-up culls are underestimates. However, the evidence for social perturbation being the underlying mechanism is not conclusive, and moreover may be one of many underlying factors truly driving the observed differences between the parentage assignment rates of the initial and follow-up culls. As mentioned previously, levels of badgers being trap-happy or trap-shy, as well as food stress levels, may also be affected by whether or not a recent badger removal operation took place. Under these alternative hypotheses, there is no reason why more parents would be likely to be captured far from the cub compared to parents of cubs

captured during the initial culls. Therefore it is unlikely there would be more captured-but-unassigned parents compared to those in the initial culls, and so would suggest that the estimates of the culling efficacies of the follow-up culls are more unbiased (than under the assumption of social perturbation being the underlying mechanism). If a combination of the above hypotheses explaining the differences in parentage assignments success between initial and follow-up culls is true, then it is likely that the culling efficacy estimates of the follow-up culls are slight underestimates.

In general, his work provides a novel method of estimating the proportion of badgers culled during each cull of the RBCT, with the results of the initial culls presented here agreeing with the proposed culling efficacy of 70% as a condition for licensing the free shooting of badgers, as used in the first year of the pilot culls in England in 2013 (DEFRA, 2013). Moreover, results from follow-up culls provide a first estimate of the culling efficacy of RBCT follow-up culls of 57.8% (CI: 51.6-64.0%). Both of these estimates can be used as guideline culling efficacies for future disease management strategies involving repeated badger culls.

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Chapter 4: Assessing the relationship between parent and cub *Mycobacterium bovis* status in badgers (*Meles meles*)

Abstract

Understanding parental influences on *Mycobacterium bovis* infection in cubs could provide significant insight into the epidemiology and possible generational persistence of the infection in badger (*Meles meles*) populations. One parental *M. bovis* transmission process that has been repeatedly hypothesised is pseudo-vertical transmission, in which *M. bovis* is transmitted from an infected mother to her cub during the first few weeks of a cub's life whilst the cub is highly dependent on its mother for nutrition. Such a transmission mechanism could heavily undermine badger vaccination strategies aimed at cubs, as newly post-emergent cubs may already be infected if such a transmission route exists. Alternatively, cubs could inherit a genetic predisposition in susceptibility to *M. bovis* infection from parents possessing particular genes. Such an inherited predisposition could come from either mother or father.

Parentage assignments generated in Chapter 3 were used in combination with *M. bovis* infection status to test mother-cub and father-cub infection associations. The probability of a cub being infected with *M. bovis* was analysed using logistic regression models, which were adjusted for *M. bovis* infection prevalence in local non-parents, as well as cub age.

Model results showed that the presence of either maternal or paternal *M. bovis* infection significantly increased the risk of cub infection ($p = 0.008$ for both). The odds ratios of the maternal and paternal infection predictors were of a similar magnitude, and there was no evidence of any additional mother-cub infection mechanism. Further model interaction terms showed no evidence for pseudo-vertical transmission, and that paternal distance had a non-significant effect on father-cub infection association.

These results suggest that both maternal and paternal infection status are equally important in cub infection risk. As mothers have far more intimate contact with their cubs than fathers, it is likely that these parent-cub infection associations are due to a genetic inheritance of *M. bovis* susceptibility rather than parent-cub transmission processes. The existence of a genetic predisposition in susceptibility to *M. bovis* would mean that many infected badgers could be

genetically highly susceptible to *M. bovis*. This would mean that selective culling or selective sterilisation could reduce the proportion of individuals highly susceptible to *M. bovis* in future generations of badgers.

However there are current practical limitations to employing such disease management strategies, as well as a need for more direct evidence of such a genetic predisposition. Suggested directions for future research are discussed.

Introduction

Badgers (*Meles meles*) are believed to become infected with *Mycobacterium bovis* predominantly via the airborne route, as most observed lesions tend to be in the lungs (Clifton-Hadley *et al.*, 1993, Nolan and Wilesmith, 1994, Jenkins *et al.*, 2008). However, badgers may also become infected through bite wounds, and it is thought that infection acquired via this route could be more fatal (Clifton-Hadley *et al.*, 1993, Jenkins *et al.*, 2008). *M. bovis* infection in badgers can persist in high-density populations for a sustained period of time (Delahay *et al.*, 2000). It has been argued that pseudo-vertical transmission (from mother to cub after birth whilst cubs are still dependent, Morris *et al.*, 1994) may contribute to the persistence of *M. bovis* infection within badger populations (Wilesmith, 1991); however there is no genetic evidence of this to date. Various mathematical models of *M. bovis* dynamics in badger populations have suggested that such a transmission route would increase the persistence of *M. bovis* infection in badger populations (Anderson and Trewhella, 1985, Smith *et al.*, 1997), and mathematical models of *M. bovis* in other wildlife species have also incorporated pseudo-vertical transmission (Zanella *et al.*, 2012). As well as direct *M. bovis* transmission events between mother and cub, any link between *M. bovis* infection in mothers and cubs could also be caused by cubs inheriting a genetic predisposition in susceptibility to *M. bovis* from their mothers.

Fathers are much less likely to transmit *M. bovis* to their cubs than mothers, as they are typically not as involved in the rearing of cubs (Neal and Cheeseman, 1996), with many fathers not even from the same social group as the cub (Dugdale *et al.*, 2007). However, cubs may inherit a genetic predisposition in susceptibility to *M. bovis* from their father.

One approach to managing *M. bovis* infection in badgers is vaccination, with injectable vaccines currently being used on wild badger populations (Chambers *et al.*, 2010). However, injectable vaccines cannot be used on cubs until they first emerge from their sett. If badger cubs are significantly more like to become infected before they have left their sett for the first time through pseudo-vertical transmission, then this may reduce the impact of any disease management programme using injectable vaccines.

An alternative solution to managing *M. bovis* infection in badgers to vaccination is badger culling. Non-selective culling is known to disrupt badgers' typically robust social structure and cause them to range more widely (termed perturbation, Pope *et al.*, 2007, Woodroffe *et*

al., 2006), causing detrimental effects to bovine TB levels in cattle (Donnelly *et al.*, 2007). Although perturbation can be a result of even a small badger population removal, it is still inconclusive whether this is always the case (Bielby *et al.*, 2014). Selective culling, in which only infected or epidemiologically important individuals (for example super-infectious badgers (Graham *et al.*, 2013, Smith *et al.*, 1995)) were culled, might avoid detrimental effects of perturbation observed in non-selective culling. If cubs could inherit a genetic predisposition in susceptibility to *M. bovis* from their parents, then selective culling may be effective at preventing future generations of individuals that are highly susceptible to *M. bovis*.

This chapter aims to assess the evidence for the following hypotheses:

- infected mothers transmit *M. bovis* infection to their cubs before they first leave their sett through pseudo-vertical transmission;
- cubs can inherit a genetic predisposition in susceptibility to *M. bovis* infection from either parent.

Methods

Data

The phenotypic data of the 8,064 badgers described in Chapter 3 were used for this work, as well as the results of both the "all culls" and "initial culls" parentage assignments generated in Chapter 3. To assess *M. bovis* infection associations, data on the binary infection status (infected [1] or uninfected [0]) of badgers were also used. Badgers were classed as infected if *M. bovis* was found in any tissue sample by bacteriological culture, or if acid-fast bacteria were found in lesions by Ziehl Neelsen staining (Gallagher and Clifton-Hadley, 2000).

Statistical analyses

"all culls"

In order to test for pseudo-vertical transmission, the infection status of cubs and their mothers were analysed using logistic regression models, with cub infection status as the binary outcome variable. Using the maternal assignment probabilities (MAPs) from the "all culls" method described in Chapter 3, two definitions of maternal infection were constructed:

- **Weighted maternal infection**:

$$\frac{\sum_{candidate\ mothers}\ (MAP)(infection\ status)}{\sum_{candidate\ mothers}\ MAP}$$

- **Modal maternal infection**: the infection status of the candidate mother with the modal MAP value (defined as the "modal mother").

However, any association between cub and mother infection status could be due to a genetic predisposition in susceptibility to *M. bovis* (and not pseudo-vertical transmission). Therefore the infection status of fathers was also included in the logistic regression models, as any association between cub and father infection status would likely point towards a genetic predisposition in *M. bovis* susceptibility as opposed to a transmission event, as fathers are not heavily involved in the rearing of cubs (Neal and Cheeseman, 1996). Using the paternal

assignment probabilities (PAPs) from the "all culls" method, two corresponding definitions of paternal infection were constructed:

- **Weighted paternal infection**:

$$\frac{\sum_{candidate\ fathers}\ (PAP)(infection\ status)}{\sum_{candidate\ fathers}\ PAP}$$

- **Modal paternal infection**: the infection status of the candidate father with the modal PAP value (defined as the "modal father").

The difference between any mother-cub and father-cub associations was also be analysed to see if evidence for both pseudo-vertical transmission and a genetic predisposition in *M. bovis* susceptibility was present.

Any observed parent-cub infection associations could also be caused by local *M. bovis* infection. Therefore two corresponding measures of *M. bovis* prevalence from other local adult females were also defined:

- **Weighted local adult female prevalence**:

$$\frac{(\sum_{local\ AF}\ infection\ status) - (\sum_{local\ AF}\ (MAP)(infection\ status))}{(number\ of\ local\ AF) - (\sum_{local\ AF}\ MAP)}$$

- **Modal local adult female prevalence**:

$$\frac{(\sum_{local\ AF}\ infection\ status) - (infection\ status\ of\ modal\ mother)}{(number\ of\ local\ AF) - 1}$$

where "local" means no further than 1km from the cub, and "AF" stands for adult females.

Two similar measures of *M. bovis* prevalence from other local adult males were also defined:

- **Weighted local adult male prevalence**:

$$\frac{(\sum_{local\ AM}\ infection\ status) - (\sum_{local\ AM}\ (PAP)(infection\ status))}{(number\ of\ local\ AM) - (\sum_{local\ AM}\ PAP)}$$

- **Modal local adult male prevalence**:

$$\frac{(\sum_{local\ AM}\ infection\ status) - (\ \delta_{local}\ F_{infected})}{(number\ of\ local\ AM) - \chi_{local}}$$

where δ_{local} equals one if the modal father is local, and zero otherwise;

$F_{infected}$ is the infection status of the modal father; and

"AM" stands for adult males.

Using cub infection status as the binary outcome variable, two logistic regression models based on the above definitions were analysed. The first of these was the "weighted" regression model:

"Weighted" model predictors:

- Weighted maternal infection,
- Weighted paternal infection,
- Weighted local adult female prevalence,
- Weighted local adult male prevalence,
- Cub age (in months).

For the "weighted" model analysis, only cubs with a null MAP (that is, the probability a cub's mother was not in the dataset) and null PAP (the probability a cub's father was not in the dataset) of less than 20% were analysed. This was to ensure only cubs that had a greater than 80% chance of each of their parents being in the dataset were analysed; an 80% cut-off value has also been used in other parentage studies (Dugdale *et al.*, 2011, Dugdale *et al.*, 2007). Furthermore, only cubs that had well-defined weighted adult local female and adult

male prevalence values (i.e. so that the denominator of these two definitions did not equal zero) were analysed. The resultant total number of cubs was 413.

The second logistic regression model was the "modal" model:

"Modal" model predictors:

- Modal maternal infection,
- Modal paternal infection,
- Modal local adult female prevalence,
- Modal local adult male prevalence,
- Cub age (in months).

For the "modal" model analysis, only cubs with a modal MAP (the MAP of the modal mother) and modal PAP (the PAP of the modal father) of greater than 80% were analysed. This was to ensure only cubs that had at least an 80% chance of each of their modal parents being in the dataset were analysed. As with the "weighted" analysis, only cubs that had well-defined modal adult female and adult male prevalence values were analysed. The resultant total number of cubs was 236.

"initial culls"

Parentage assignments generated using the "initial culls" method (for cubs taken during the initial proactive culls of the RBCT, as described in Chapter 3) were used for these analyses, as were the social group allocations outlined in Chapter 3 (taken from Woodroffe *et al.*, 2009). Of the 405 cubs captured during the initial culls, only the 373 cubs that were allocated a unique social group (Woodroffe *et al.*, 2009) were analysed. Each adult was then given a "group weighting" for each social group they were allocated to, equivalent to the reciprocal of the number of groups they were allocated to in total. For example, an adult allocated to two social groups was given a social group weighting of 0.5 for each of the two social groups it was allocated to.

The same definitions for weighted/modal maternal/paternal infection as used in the "all culls" analyses above were applied in these analyses too. Similar measures of local adult female prevalence were defined for these analyses, however as social group allocations were also available for individuals in this dataset, only adult females with a non-zero "group weighting"

(GW) value for the same social group as the cub were considered. The two measures for *M. bovis* prevalence in other same-group adult females were defined as:

- **Weighted group adult female prevalence**:

$$\frac{\left(\sum_{group\ AF}\ (infection\ status)(GW)\right) - \left(\sum_{group\ AF}\ (MAP)(infection\ status)(GW)\right)}{\left(\sum_{group\ AF}\ GW\right) - \left(\sum_{group\ AF}\ (MAP)(GW)\right)}$$

- **Modal group adult female prevalence**:

$$\frac{\left(\sum_{goup\ AF}\ (infection\ status)(GW)\right) - (GW_{modal}\ M_{infected})}{\left(\sum_{group\ AF}\ GW\right) - GW_{modal}}$$

where GW_{modal} is the group weighting of the modal mother;

$M_{infected}$ is the infection status of the modal mother.

As almost half of fathers are thought to not be from the same social group as the cub (Dugdale *et al.*, 2007), measures of local adult male prevalence were based on adult males within 1km of the cub, as in the "all culls" analyses. Therefore the same definitions for weighted and modal local adult male prevalence as used for the "all culls" analyses were also used for these "initial culls" analyses.

Two corresponding "weighted" and "modal" logistic regression models using the above "initial culls" definitions were analysed:

"Weighted" model predictors:

- Weighted maternal infection,
- Weighted paternal infection,
- Weighted group adult female prevalence,
- Weighted local adult male prevalence,
- Cub age (in months).

As in the "all culls" analyses, only cubs with a null MAP (the probability a cub's mother was not in the dataset) and null PAP (the probability a cub's father was not in the dataset) of less than 20% were analysed for the "weighted" model. Furthermore, only cubs that had well-defined weighted group adult female and weighted local adult male prevalence values were analysed. The resultant total number of cubs for this analysis was 191.

The second logistic regression model was the "modal" model:

"Modal" model predictors:

- Modal maternal infection,
- Modal paternal infection,
- Modal group adult female prevalence,
- Modal local adult male prevalence,
- Cub age (in months).

For this "modal" model analysis, only cubs with a modal MAP (the MAP of the modal mother) and modal PAP (the PAP of the modal father) of greater than 80% were analysed. Again, only cubs that had well-defined modal adult female and adult male prevalence values were analysed. The resultant total number of cubs for this analysis was 108.

Interaction models

An additional consideration in assessing whether pseudo-vertical transmission of *M. bovis* takes place is in analysing the age at which cubs become infected. If there is evidence that cubs are more likely to become infected with *M. bovis* earlier rather than later on during their first year of life, it may suggest that there is evidence for pseudo-vertical transmission, as cubs are more likely to have more intimate contact with their mothers during their first few months of life (Neal and Cheeseman, 1996). By the nature of the data collected, only post-emergent cubs were captured, and thus may have been almost or fully independent of their mothers by this time. However, the onset of clinical signs of *M. bovis* infection in a newly infected badger is known to be delayed by an incubation period. Little *et al.* (1982) observed that the incubation period of *M. bovis* infection in badgers could last between three to five months, and so even cubs that were infected with *M. bovis* via a pseudo-vertical transmission route prior to first emerging from their sett might only have a detectable level of *M. bovis* from May onwards. Therefore interaction terms between maternal infection status and cub

age were also analysed in all the above regression models to look for any evidence of higher rates of infection transmission to cubs before they first emerge from their sett (through pseudo-vertical transmission).

A second interaction term that was investigated was based on father-cub distance. As mentioned above, fathers are not heavily involved in rearing cubs (Neal and Cheeseman, 1996), however they may be the most likely adult male that the cub has contact with if the father is nearby. As males are more likely to be excretors of *M. bovis* (Graham *et al.*, 2013), it could be possible that any observed association between father and cub infection status is due to an *M. bovis* transmission event from nearby fathers (instead of a genetic predisposition in susceptibility). This hypothesis was tested by looking at the interaction between father-cub distance and paternal infection. For the "modal" analyses, the "modal father-cub distance" was defined to be the distance between the cub and the modal father. For the "weighted" analyses, the "weighted father-cub distance" was defined as:

$$\frac{\sum_{candidate\ fathers}\ (PAP)(paternal\ distance)}{\sum_{candidate\ fathers}\ PAP}$$

where $paternal\ distance$ is the distance between the cub and the candidate father.

Sensitivity analysis

All analyses were also performed using MAP and PAP cut-off values of 50%, 60%, 70% and 90%, to assess the sensitivity of the assumption of an 80% cut-off value.

Results

"all culls"

Table 4.1 shows the odds ratio estimates (with 95% confidence intervals [CI]) and the p-values of the five predictor variables for the "weighted" regression model. Both maternal (p = 0.013) and paternal (p = 0.025) infection were significantly positively associated with cub infection status, as was cub age (p = 0.006). Both weighted local adult female prevalence (p = 0.090) and weighted local adult male prevalence (p = 0.291) were non-significant in the model, however the weighted local adult female prevalence variable was included in the reduced model (Table 4.2) as its p-value was below 0.10. Only terms that were significant in the full "weighted" model (Table 4.1) were significant in the reduced "weighted" model (Table 4.2).

The interaction of weighted maternal infection with cub age was added to the reduced model of Table 4.2, and the p-value was non-significant (p = 0.559). Similarly, the interaction of weighted paternal infection with weighted father-cub distance (defined in the methods section) was also added to the reduced model, and the p-value was also non-significant (p = 0.109).

Table 4.3 shows the odds ratio estimates (with 95% CI) and the p-values of the five predictor variables for the "modal" regression model. As with the "weighted" analyses, both maternal (p = 0.028) and paternal (p = 0.004) infection were significantly positively associated with cub infection status for the "modal" analyses, as again was cub age (p = 0.010). Both modal local adult female prevalence (p = 0.075) and modal local adult male prevalence (p = 0.274) were non-significant, however as in the "weighted" analyses, the local adult female prevalence term was included in the reduced model as its p-value was below 0.10. All terms in the reduced "modal" model (Table 4.4) were now significantly positively associated with cub infection status.

The interaction of modal maternal infection with cub age was added to the reduced model of Table 4.4, and the p-value was non-significant (p = 0.667). The interaction of modal paternal infection with modal father-cub distance was also added to the reduced model, resulting in a marginally non-significant negative association (p = 0.059).

Table 4.1: Co-efficient estimates (with 95% CI) and p-values for the full "weighted" logistic regression model based on the "all culls" data.

Predictor variable	Odds ratio (95% CI)	p-value
Weighted maternal infection	5.884 (1.446-23.938)	0.013
Weighted paternal infection	3.852 (1.188-12.491)	0.025
Weighted local adult female prevalence	7.067 (0.739-67.573)	0.090
Weighted local adult male prevalence	2.786 (0.415-18.699)	0.291
Cub age (in months)	1.408 (1.102-1.800)	0.006

Table 4.2: Co-efficient estimates (with 95% CI) and p-values for the reduced "weighted" logistic regression model (with only predictor variables that were significant or nearly significant in Table 4.1 above) based on the "all culls" data.

Predictor variable	Odds ratio (95% CI)	p-value
Weighted maternal infection	6.553 (1.637-26.232)	0.008
Weighted paternal infection	4.581 (1.480-14.183)	0.008
Weighted local adult female prevalence	6.865 (0.759-62.057)	0.086
Cub age (in months)	1.430 (1.121-1.824)	0.004

Table 4.3: Co-efficient estimates (with 95% CI) and p-values for the full "modal" logistic regression model based on the "all culls" data.

Predictor variable	Odds ratio (95% CI)	p-value
Modal maternal infection	7.131 (1.233-41.219)	0.028
Modal paternal infection	8.300 (2.002-34.408)	0.004
Modal local adult female prevalence	13.701 (0.768-244.414)	0.075
Modal local adult male prevalence	4.159 (0.324-53.379)	0.274
Cub age (in months)	1.628 (1.126-2.353)	0.010

Table 4.4: Co-efficient estimates (with 95% CI) and p-values for the reduced "modal" logistic regression model (with only predictor variables that were significant or nearly significant in Table 4.3 above) based on the "all culls" data.

Predictor variable	Odds ratio (95% CI)	p-value
Modal maternal infection	9.220 (1.702-49.936)	0.010
Modal paternal infection	8.617 (2.107-35.243)	0.003
Modal local adult female prevalence	15.883 (1.078-234.011)	0.044
Cub age (in months)	1.639 (1.147-2.342)	0.007

"initial culls"

Only 192 cubs had parentage assignments that met the criteria for being included in the "weighted" logistic regression analyses, of which only 9 tested positive for *M. bovis*. Furthermore, only 108 cubs had parentage assignments that met the criteria for being included in the "modal" logistic regression analyses, of which only 7 tested positive for *M. bovis*. Therefore these low levels of cub infection did not allow any accurate estimates of maternal or paternal infection association so as to be informative.

Sensitivity analysis

Table 4.5 shows the odds ratios (with 95% CI) and p-values for corresponding "weighted" regression models using MAP and PAP cut-off values of 50%, 60%, 70%, 80% (the same as results in Table 4.1) and 90%. Weighted maternal infection was significantly associated with cub infection status in all models except when a 90% cut-off value was used ($p = 0.077$), and weighted paternal infection was significantly associated with cub infection status for all models.

Table 4.6 gives the same sensitivity analysis results for the "modal" regression models. Only the original 80% model gave an initial significant association between modal maternal infection and cub infection status, however when the most non-significant term of the model assuming a cut-off of 70% was removed (modal local adult female prevalence, $p = 0.220$), there was a significant association between modal maternal infection and cub infection status ($p = 0.046$). Modal paternal infection was significantly associated with cub infection status in all models except when a 90% cut-off value was used ($p = 0.084$).

The two interaction terms defined earlier were also tested for all "weighted" and "modal" models. The only significant interaction term came from the "weighted" model using a 90% cut-off value, which found an association between the interaction of weighted father-cub distance and weighted paternal infection with cub infection status ($p = 0.039$).

Table 4.5: Co-efficient estimates (with 95% CIs) and p-values for the full "weighted" logistic regression models based on the "all culls" data, assuming cut-off values of 50, 60, 70, 80 and 90%.

	MAP and PAP cut-off value (%)									
	50		**60**		**70**		**80**		**90**	
Predictor variable	*OR (95% CI)*	*p*	*OR (95% CI)*	*p*	*OR (95% CI)*	*p*	*OR (95% CI)*	*p*	*OR (95% CI)*	*p*
Weighted maternal infection	4.075 (1.121, 14.807)	0.033	5.246 (1.446, 19.029)	0.012	7.228 (1.905, 27.422)	0.004	5.884 (1.446, 23.938)	0.013	4.701 (0.846, 26.119)	0.077
Weighted paternal infection	3.764 (1.341, 10.563)	0.012	3.084 (0.998, 9.532)	0.050	3.431 (1.083, 10.869)	0.036	3.852 (1.188, 12.491)	0.025	4.806 (1.268, 18.221)	0.021
Weighted local adult female prevalence	5.374 (0.740, 39.031)	0.096	13.847 (1.963, 97.680)	0.008	8.474 (1.023, 70.218)	0.048	7.067 (0.739, 67.573)	0.090	12.095 (1.127, 129.795)	0.040
Weighted local adult male prevalence	6.251 (1.555, 25.123)	0.010	4.731 (0.918, 24.386)	0.063	3.675 (0.642, 21.025)	0.144	2.786 (0.415, 18.699)	0.291	1.815 (0.194, 17.023)	0.602
Cub age (in months)	1.442 (1.183, 1.759)	<0.001	1.433 (1.155, 1.779)	0.001	1.388 (1.108, 1.737)	0.004	1.408 (1.102, 1.800)	0.006	1.502 (1.151, 1.960)	0.003

Table 4.6: Co-efficient estimates (with 95% CIs) and p-values for the full "modal" logistic regression models based on the "all culls" data, assuming cut-off values of 50, 60, 70, 80 and 90%.

	MAP and PAP cut-off value (%)									
	50		**60**		**70**		**80**		**90**	
Predictor variable	*OR (95% CI)*	*p*	*OR (95% CI)*	*p*	*OR (95% CI)*	*p*	*OR (95% CI)*	*p*	*OR (95% CI)*	*p*
Modal maternal infection	3.061 (0.845, 11.093)	0.089	2.595 (0.612, 10.999)	0.196	4.152 (0.866, 19.896)	0.075	7.131 (1.233, 41.219)	0.028	5.338 (0.275, 103.636)	0.268
Modal paternal infection	3.589 (1.215, 10.604)	0.021	5.290 (1.684, 16.622)	0.004	6.448 (1.878, 22.137)	0.003	8.300 (2.002, 34.408)	0.004	5.962 (0.787, 45.142)	0.084
Modal local adult female prevalence	13.596 (1.583, 116.776)	0.017	14.549 (1.593, 132.879)	0.018	6.433 (0.329, 125.762)	0.220	13.701 (0.768, 244.414)	0.075	79.414 (2.327, 2709.932)	0.015
Modal local adult male prevalence	14.237 (2.913, 69.574)	0.001	7.798 (1.346, 45.186)	0.022	6.178 (0.688, 55.497)	0.104	4.159 (0.324, 53.379)	0.274	2.406 (0.062, 92.971)	0.638
Cub age (in months)	1.449 (1.138, 1.845)	0.003	1.509 (1.153, 1.975)	0.003	1.552 (1.123, 2.144)	0.008	1.628 (1.126, 2.353)	0.010	1.817 (1.077, 3.067)	0.025

Discussion

The results of the "all culls" analyses showed that both increased maternal and paternal infection (using both the "weighted" and "modal" definitions of maternal and paternal infection) was significantly associated with increased cub infection status. In both the "weighted" and "modal" analyses, the maternal and paternal infection odds ratios were not significantly different, and thus there was no detectable increase in infection risk to cubs from having an infected mother compared to having an infected father (such an increased risk might have suggested an additional maternal mechanism driving mother-cub infection association, such as pseudo-vertical transmission).

It could have been possible that the similar magnitude of the maternal and paternal odds ratios were due to close contact from both parents, as well as a genetic predisposition from both parents. However the non-significant interactions of paternal infection and father-cub distance in both the "weighted" and "modal" analyses suggest that there is no epidemiologically important close father-cub contact. Furthermore, the non-significant interactions of maternal infection and cub age for both the "weighted" and "modal" analyses would suggest that cubs are no more likely to become infected when younger, and so provide no evidence for pseudo-vertical transmission. The non-significance of these interaction terms, together with the similar magnitude in the odds ratios of maternal and paternal infection, suggest that cubs may inherit a genetic predisposition is susceptibility to *M. bovis* infection, and that there is no evidence that parent-cub transmission events are driving any of the observed parent-cub infection associations.

An increase in local adult female prevalence was significantly associated with increased cub infection status in the "modal" analyses, but not for the "weighted" analyses. Using the fact that the mean number of local non-mother adult females from the "modal" dataset was 7.729, the estimated increase in the odds of a cub being infected due to the presence of an infected local non-mother adult female was found to be 1.430 (95% CI: 1.010-2.026), based on the modal local adult female prevalence odds ratio estimate of 15.883 (95% CI: 1.078-234.011). Compared to the maternal infection odds ratio estimate of 9.220 (95% CI: 1.702-49.936), the per-local-infected-adult-female odds ratio estimate was significantly lower ($\chi^2 = 4.485$, df = 1, p = 0.034). Therefore although the "modal" analyses suggest that local adult females play an important role in determining cub infection status, the importance of the mother is significantly greater.

The corresponding estimate for the increase in the odds of a cub being infected due to the presence of an infected local non-mother adult female based on the "weighted" dataset (for which the mean number of local non-mother adult females was 9.103) was 1.236 (95% CI: 0.970-1.574) based on the weighted local adult female prevalence odds ratio estimate of 6.865 (95% CI: 0.759-62.057). As with the "modal" analyses, the maternal odds ratio estimate was significantly greater than the per-local-infected-adult-female odds ratio estimate ($\chi^2 = 5.391$, df = 1, p = 0.020).

The "weighted" analyses gave a non-significant association between cub infection status and local adult female prevalence, whereas the "modal" analyses gave a significant association. By its nature, the "weighted" analyses accounts for more of the information in the parentage data (through considering partial parentage) and thus allows more cubs with high-confidence parentage assignments to be analysed than the "modal" analyses. Furthermore, the per-local-infected-adult-female odds ratio was significantly lower than the maternal odds ratio for both analyses. Therefore the conclusion is that the association between cub infection status and local adult female prevalence is borderline non-significant.

The sensitivity analysis indicated that the MAP and PAP cut-off values used for the "weighted" models did not affect whether maternal or paternal infection was significantly associated with cub infection status, except when a cut-off of 90% was used. This is likely to be due to lower numbers of cubs with a null MAP and null PAP of less than 10% (359 compared to 413 used in the analysis using a cut-off value of 80%). The "modal" models only gave a significant association between maternal infection and cub infection status when a cut-off value of 70% or 80% was applied. Models using the lower MAP and PAP cut-off values of 50% and 60% will have more cubs with the wrong parents assigned, thus dampening any true underlying effect.

The single significant interaction term observed in the model using a 90% cut-off could potentially mean that an association between father and cub infection status is due to proximity, and thus possibly a transmission event. However, given that the distance between a cub and a candidate father is taken into account when assigning paternity, it could also be that this is driving such an interaction when only high-valued PAPs (i.e. greater than 90%) are considered.

As expected, increased cub age was also significantly associated with increased cub infection status, with the odds of a cub being infected estimated to increase by 1.430 (95% CI: 1.121-

1.824) per month under the "weighted" analyses, and 1.639 (95% CI: 1.147-2.342) per month under the "modal" analyses.

The evidence presented in this chapter for cubs inheriting a genetic predisposition in susceptibility to *M. bovis* infection from their parents raises many interesting questions about how to manage *M. bovis* in infected badger populations. If most badgers infected with *M. bovis* have a genetic susceptibility to *M. bovis* infection, then attempting to cull infected individuals may reduce the number of individuals that are highly susceptible to *M. bovis* infection in future generations of badgers.

There are currently several issues with culling strategies based on targeting only infected individuals, known as 'selective culling', in which badgers would be trapped, tested for *M. bovis*, and culled if found to be infected. Firstly, there is no effective live test for detecting *M. bovis* in badgers, with current live test sensitivity estimated to be between 50% and 80% (Drewe *et al.*, 2010). Therefore there would be many infected badgers testing negative for *M. bovis* and consequently being released. Secondly, some badgers will always be missed in a culling operation, including infected badgers. Although large-scale culling is known to cause changes in badger behaviour that can have detrimental effects to bovine TB levels in cattle if *M. bovis* is present in the surrounding badger population, there is also evidence that culling a smaller proportion of a badger population may induce similar behaviour (Bielby *et al.*, 2014). However, Bielby *et al.* (2014) found that small-scale culling did not always cause severe perturbation in badger populations, and so it is possible that the benefits of selectively culling badgers that may be highly susceptible to *M. bovis* infection could outweigh the detrimental effects of selective (and thus only targeting a small proportion of the population, assuming a low *M. bovis* prevalence) culling on badger behaviour. However such a strategy is only likely to work if an effective live test is developed, which may be several years away.

Alternatively, sterilising infected badgers may also prevent future generations of many highly susceptible individuals, whilst avoiding any potentially detrimental effects of selective culling to badger behaviour. This would work on the same assumption that infected badgers may be likely to have a genetic predisposition in *M. bovis* susceptibility, and so sterilising these individuals may reduce the frequency of highly susceptible individuals in future generations. Although sterilisation of wildlife host species has been used as a disease management strategy elsewhere (Ramsey, 2000, Ramsey *et al.*, 2006), the idea of sterilising badgers has rarely been considered as a viable option for bovine TB control in the UK

(Tuyttens and MacDonald, 1998). This is likely to be because a non-selective sterilisation programme would have a huge impact on badger population structure. In contrast, a selective sterilisation programme based on only sterilising individuals infected with *M. bovis* would be likely to have a much smaller impact on population structure. However, this would still rely on an effective live test for *M. bovis* in order for such a disease management strategy to be applied optimally.

Although this work does provide some evidence of a genetic predisposition in susceptibility to *M. bovis* infection in badgers, this evidence presented here is not conclusive. Further work is required to generate more direct evidence of the presence of this epidemiological mechanism before any of the implementation of the possible disease management strategies suggested above could be seriously considered. Similar work has looked at associations between classes of genes and susceptibility to certain pathogens in badgers (Sin *et al.*, 2014), as well as genetic *M. bovis* predisposition in other animals (Ladel *et al.*, 1995).

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Chapter 5: Analysis of the RBCT closed season on badger (*Meles meles*) cub welfare

Abstract

During the RBCT, a three-month period between February and April inclusive known as the 'closed season' was imposed, during which no culling of badgers (*Meles meles*) took place. This was designed to prevent the culling of mothers of cubs who had not yet emerged from their setts, and thus were still nutritionally dependent on their mothers. The timing of this period was chosen because the majority of badger cubs are born in February each year, and usually do not emerge from their setts until 6-8 weeks after birth.

To test whether this three-month period was sufficiently long in duration, parentage assignments were analysed to identify likely mothers of cubs, and thus calculate the mean number of culled cubs per mother from each RBCT cull. The mean of these values for each month was compared to expected values based on cub survival estimates, generated using field data from Wytham Woods, Oxfordshire.

The mean number of culled cubs per mother observed for May was not lower than expected under any assumption of RBCT cub trappability. Therefore there was no evidence to suggest that dependent cubs of culled mothers were missed during the RBCT proactive culls, and therefore no evidence that a longer closed season is required. However, more direct evidence is needed in order to definitively rule out the possibility that many whole litters are being missed using such a closed season.

Introduction

Badger welfare

Badgers (*Meles meles*) are a protected species in the UK under the Protection of Badgers Act 1992, and in the planning of most culling operations badger welfare is usually an important consideration. There has been much research looking into the welfare of badgers during culling operations (Ewbank, 2003, Kirkwood, 2000, MAFF, 1983), including research looking specifically into the welfare of trapped badgers (Woodroffe *et al.*, 2005a, Woodroffe *et al.*, 2005b); there are also charitable organisations such as The Badger Trust whose work is mainly focussed towards badger welfare.

Possible direct effects of cage trapping on badger welfare include whether badgers die rapidly when shot (which is also a major issue with the proposed method of free shooting outlined in Chapter 1), levels of distress caused from being trapped in a cage, as well as physical stress on claws and teeth from attempting to dig or bite the cage whilst trapped (Woodroffe *et al.*, 2005b).

Badger culling can also affect the welfare of cubs dependent on mothers which are killed. Badger cubs are mostly born in February (Neal and Cheeseman, 1996), and although other non-mother adult females from the same social group can assist in juvenile care (known as alloparental care, Dugdale *et al.*, 2010), cubs are nutritionally dependent on their mothers for a period of between 6-8 weeks (Neal and Cheeseman, 1996). If the mother of a cub is culled during this period, the cub could die from starvation or dehydration in their underground sett, amounting to considerable suffering to the cub.

One previous approach to minimise the impact of culling on badger cub welfare involved the release of lactating females that were cage-trapped, as their active lactation status was thought to have indicated that they may have dependent cubs (Krebs *et al.*, 1997). However, this method proved problematic. Culling operations that took place between 1996 and 1998 found that 4.3% of cage-trapped adult females were later found to have an active lactation status during post-mortem examination (Woodroffe *et al.*, 2005a), thus highlighting the difficulty of assessing the lactation status of an adult female without removing it from the cage.

The RBCT closed season

During the RBCT, a 3-month period called the "closed season" running from February to April inclusive was implemented, during which no culling took place. This was developed with the aim of minimising the possibility of culling mothers with dependent cubs, whilst not compromising the overall aim of having an effective culling strategy. When details of the plans for the closed season were made public, the National Federation of Badger Groups (NFBG – now the Badger Trust) estimated that around 2,300 badger cubs would be left to die in their underground setts during the first round of culls in the RBCT as a consequence of killing their mothers, even when implementing the closed season (NFBG, 1999). However, subsequent work matching lactating females to cubs based on capture location found this figure to be a substantial overestimate, and concluded that a maximum of approximately 9 cubs per year would be left to starve (Woodroffe *et al.*, 2005a). One limitation of this previous work however is that cubs may still be dependent on their mothers for a period after their mothers have stopped lactating, for example whilst learning to forage, and so mothers of dependent cubs may not be lactating. Another problem of previous work lies in the difficulty of assigning mothers and cubs based on location, for example a mother may be visiting a neighbouring sett when captured. In order to confidently estimate how many dependent cubs of culled mothers were not culled, there needs to be an improved method of assessing whether a female had dependent cubs that year, and in mother-cub assignments. The use of genetic data for generating more confidence in this estimate has been suggested in previous work (Woodroffe *et al.*, 2005a), but has not yet been undertaken.

The main focus of this chapter is to assess whether the closed season from the RBCT was sufficiently long enough to minimise the number of badger cubs dying in their setts due to their mothers being culled whilst the cubs are still dependent, through the use of genetic parentage assignments.

Methods

Data

The phenotypic data of the 8,064 badgers used in Chapters 3 and 4 were also used in this chapter, as were the "all culls" parentage assignments generated in Chapter 3. The "initial culls" parentage assignments were not used in this chapter, as cub data from just the initial culls was too limited to be of use (for example, no initial cull took place during June or August).

Cub capture-recapture data collected from Wytham Woods, Oxfordshire, from May to October 1990-1992 inclusive (Woodroffe, 1992) were used to estimate cub survival rates. The data was collected from a long-term study in which badgers were repeatedly captured, with no culling taking place.

Number of culled cubs per mother

Using the "all culls" parentage assignments, a maternal assignment probability (MAP) threshold was applied such that cubs assigned a modal mother with a MAP less than or equal to 80% were not used in subsequent analyses, as used in similar genetic parentage work (Dugdale *et al.*, 2007). MAP values greater 80% will subsequently be defined as being a "qualifying MAP"; similarly cubs with a qualifying MAP will be referred to as being a "qualifying cub". Using this reduced dataset, the mean number of culled cubs per mother based on assigned modal mothers for qualifying cubs were calculated and categorised by month of cull. The mean number of culled cubs per mother for each month was then calculated.

To account for culls that took place during two or more calendar months (Table 5.1), the mean number of culled cubs per mother for such a cull was partially assigned to each month the cull took place, based on the proportion of cull days in each of these months. In general, if we let:

d_{ij} be the number of days cull i took place during month j;

D_i be the number of days cull i took place over all months (that is $D_i = \sum_j d_{ij}$);

c_{ik} be the number of culled cubs assigned to the k^{th} mother from cull i;

m_i be the number of mothers (assigned at least one culled cub) from cull i;

then:

$$Average\ number\ of\ culled\ cubs\ per\ mother\ for\ month\ j = \frac{\sum_i \frac{d_{ij}}{D_i}(\sum_k c_{ik})}{\sum_i \frac{d_{ij}}{D_i}(m_i)}$$

These values were calculated for each month from May to October inclusive. Weighted standard error (SE) values for the mean number of culled cubs per mother for each month were calculated using the following formula:

$$SE\ of\ average\ litter\ size\ for\ month\ j = \sqrt{\frac{s_0 s_2 - {s_1}^2}{{s_0}^2(s_0 - 1)}}$$

where:

$$s_n = \sum_i \frac{d_{ij}}{D_i}(\sum_k {c_{ik}}^n)$$

Expected number of culled cubs per mother

To estimate the expected number of culled cubs per mother by month, 10,000 post-emergent litters were simulated based on a litter size distribution taken from Neal and Cheeseman (1996), with the probability of a litter size of 1, 2, 3, 4 or 5 defined as being 0.11, 0.52, 0.29, 0.06 and 0.02 respectively, giving a mean post-emergent litter size of 2.36.

These simulated litters were then subjected to three levels of stochastic reductions based on modelling the following processes:

1. Monthly cub survival rates;
2. RBCT cub trappability;
3. Probability of having a mother assigned with a qualifying MAP.

1. Monthly cub survival rates

Monthly cub survival rates were estimated using cub capture-recapture data from Wytham Woods, Oxfordshire between 1990-1992 (Woodroffe, 1992). These were estimated by fitting a Cormack-Jolly-Seber model (Lebreton *et al.*, 1992) to the cub capture-recapture data using program MARK (version 7.1, White and Burnham, 1999) to estimate Bernoulli survival probabilities for cubs for the months of May-October inclusive. The structure of the survival model is outlined in Figure 5.1. For each month m_i, each ϕ_i represents the conditional probability of surviving to the next month given they survived until month m_i. For example, ϕ_1 represents the probability of a cub surviving until June given they survived until May. These survival parameters were estimated using maximum likelihood. To illustrate the maximum likelihood model, consider the following cub history:

100110

In relation to Figure 5.1, this means that the cub was captured in May, August and September, and not captured in June, July and October. The contribution of this cub's history to the likelihood would therefore be:

$$p\varphi_1(1-p)\varphi_2(1-p)\varphi_3 p\varphi_4 p\varphi_5[(1-p)\varphi_6 + (1-\varphi_6)]$$

where p is the probability of a cub being trapped during any month.

The final square bracket accounts for the fact that the reason for the final "0" in the cub's history is unknown: the cub could have either survived to October and not been captured, or if could have died before October. Given the presence of the fifth "1" in the cub's history, it can be inferred that the cub must have survived all previous months, and hence why all previous ϕ_i values were included in the likelihood (and not $(1-\phi_i)$).

Assuming different values of p, the values of ϕ_i were estimated so as to maximise the product of each cub's likelihood in the data. The value of p was estimated heuristically, and outlined below. The survival parameters of this model were estimated using maximum likelihood; however, other parameters of the model were estimated heuristically, as explained below.

Although MARK is capable of estimating both survival and trappability simultaneously, survival estimates are likely to be more accurate if trappability rates are fixed at

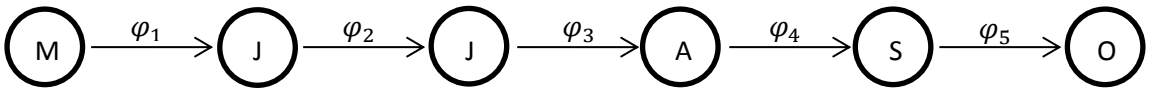


Figure 5.1: Model structure of the conditional survival probabilities between May and October.

reasonable values within the model. Tuyttens *et al.* (1999) showed that cub trappability can be significantly dependent on the badger population in question, and although they estimated cub trappability using data from Wytham Woods in their work, these estimates were based on data from a study period later than Woodroffe's (1992) work, when traps were not pre-baited (which they suggest is an important factor in the trappability of cubs). Tuyttens *et al.* (1999) also found that when traps were pre-baited, trappability was always at least 49%. Macdonald and Newman (2002) estimated annual apparent cub survival at 69.7% based on Wytham Woods data between 1990-1992, and so cub trappability values (in Wytham Woods) of 50%, 60%, 70%, 80%, 90% and 100% were trialled in the Cormack-Jolly-Seber model described above to obtain a cumulative survival of at most 69.7% by October (this assumes survival between October-January is comparatively high (Fowler and Racey, 1988, Macdonald *et al.*, 2010), as during this time cubs are completely independent of their mothers and have survived the potentially dry summer months (Woodroffe and Macdonald, 2000)). For all cub trappability values greater than 50%, the model estimated the probability of a post-emergent cub surviving until October as being less than the 69.7% annual survival rate observed in Macdonald and Newman (2002). However when a trappability value of 50% was assumed, the model estimated the probability of a post-emergent cub surviving until October as 70.5%; therefore the monthly cub survival values associated with this model were used as the final values.

2. RBCT cub trappability

Tuyttens *et al.* (1999) showed that cub trappability could be substantially affected by recent culling events, and so RBCT cub trappability rates could not be reliably inferred from the Wytham Woods cub trappability rates estimated above.

By the nature of the RBCT, badgers were captured at most once, and so estimating cub trappability of RBCT cubs is not straight forward. However, mean adult trappability over all RBCT culls has been estimated at approximately 55.7% using parentage-based approaches outlined in Chapter 3, and field studies elsewhere in England found that when traps are pre-baited (as in the RBCT) cub trappability was higher than adult trappability (Tuyttens *et al.*, 1999). Therefore five values of 60%, 70%, 80%, 90% and 100% were used to model the Bernoulli probability of trapping a cub.

3. Probability of having a mother assigned with a qualifying MAP

Once each simulated litter had undergone monthly stochastic survival and trappability events, each remaining cub underwent a third and final Bernoulli trial based on the probability of being assigned a mother with a qualifying MAP. This extra probabilistic trial was included in order to mirror the process of applying a threshold criterion of 80% on cubs' MAP values, as choosing a subset of cubs from a dataset of maternal assignments is equivalent to applying a random binomial reduction, causing potential litter size values to decrease.

The proportion of culled cubs that had a qualifying MAP was calculated for each month, and was used as the parameter value of the Bernoulli trial (denoted "probability of qualifying"). To account for culls that were performed across two or more calendar months (Table 5.1), weighted values were generated (similarly to previous calculations in this chapter) using the following formula:

$$probability\ of\ qualifying\ for\ a\ cub\ culled\ in\ month\ j = \frac{\sum_i \frac{d_{ij}}{D_i}(q_i)}{\sum_i \frac{d_{ij}}{D_i}(c_i)}$$

where: q_i is the number of cubs from cull i with a qualifying MAP;

c_i is the total number of cubs from cull i.

Once these three Bernoulli trials were performed on all of the simulated litters, the mean remaining simulated number of cubs per mother was calculated for each month (excluding litters with no cubs left) and taken to be the expected mean number of culled cubs per mother for that month. These values were compared with the observed mean number of culled cubs per mother for each month.

Table 5.1: Month and year (e.g. the 1998-1999 row runs from May 1998 to January 1999) of each RBCT cull (Bourne *et al.*, 2007) using the "triplet"-"cull number" notation. * indicates that the cull took place over more than one month.

Year	Month								
	May	**Jun**	**Jul**	**Aug**	**Sep**	**Oct**	**Nov**	**Dec**	**Jan**
1998-1999								B-1	
1999-2000						C-1	B-2*	B-2*	A-1
2000-2001	E-1		F-1	B-3*	B-3*	G-1*, B-3*	G-1*, B-3*	H-1, B-3*	C-2, E-2, B-3*
2002-2003	A-2, F-2	H-2*, E-3*	G-2, H-2*, E-3*	C-3*, E-3*	I-1*, C-3*, E-3*	I-1*, J-1, C-3*, E-3*	C-3*, E-3*, B-4*	D-1, E-3*, B-4*	E-3*
2003-2004	D-2	E-4, B-5	J-2*, G-3	J-2*	I-2*, H-3	I-2*, C-4	A-3	F-3	
2004-2005	A-4, H-4	G-4, C-5	E-5, B-6*	B-6*	D-3, F-4	I-3*, I-4*	I-3*, I-4*		
2005-2006	D-4, J-4	F-5, G-5	I-4, H-5*	H-5*	C-6, E-6	A-5, B-7			

Table 5.2: The mean number of culled cubs per mother (number of mothers) for each cull by RBCT triplet and cull number.

Triplet	Initial cull	Second cull	Third cull	Fourth cull	Fifth cull	Sixth cull	Seventh cull
A		1.67 (24)	1.25 (4)	2.60 (5)	1.00 (1)		
B			2.00 (1)	1.00 (1)	1.19 (16)	1.38 (8)	1.00 (1)
C		1.00 (1)	1.70 (10)	1.27 (11)	1.33 (12)	1.20 (5)	
D	1.00 (3)	1.47 (34)	1.08 (12)	1.89 (9)			
E	1.53 (62)	1.00 (4)	1.00 (4)	1.56 (16)	1.17 (6)	1.80 (5)	
F	1.29 (35)	1.41 (22)		1.00 (8)	1.30 (10)		
G	1.00 (7)	1.40 (20)	1.00 (9)	1.44 (9)	1.67 (9)		
H	1.25 (4)	1.41 (27)	1.00 (4)	1.33 (3)	1.00 (2)		
I	1.36 (11)	1.40 (15)	1.00 (2)	1.45 (11)			
J	1.13 (16)	1.37 (19)		1.60 (5)			

Results

Parentage analyses

Of the 1,611 cubs analysed, 1,064 were assigned a mother, with 702 of these assignments have a qualifying MAP.

Number of culled cubs per mother

Table 5.2 shows the mean number of culled cubs per mother for each cull. No cubs from culls B-1 or J-3 (using the "triplet"-"cull number" notation) had a qualifying MAP, and so no estimates of mean number of culled cubs per mother were generated for these culls. Cull A-4 had the markedly highest mean number of culled cubs per mother of 2.60. Thirteen culls had a minimal mean number of culled cubs per mother value of 1.00 (A-5, B-4, B-7, C-2, D-1, E-2, E-3, F-4, G-1, G-3, H-3, H-4, I-3), however there were fewer than ten known mothers analysed for each of these culls. No culls took place solely in August (and only six culls partially took place in this month, Table 5.1), resulting in the large confidence interval for the estimate of the mean number of culled cubs per mother in August, (Table 5.3). With the exception of August, the monthly mean number of culled cubs per mother decreased over time. The mean of the mean number of culled cubs per mother over all culls was 1.33, with a median value of 1.30.

Monthly cub survival rates

Monthly cub survival estimates using data from Wytham Woods are shown in Table 5.4. Overall cub survival was high for May, June and July, but was substantially lower August, September and October. Confidence intervals for monthly cumulative survival estimates were computed using the conditional month-by-month survival probability estimates generated by MARK (White and Burnham, 1999) by using the delta method (Oehlert, 1992).

Probability of having a mother assigned with a qualifying MAP

The proportion of cubs assigned a mother with a qualifying MAP for May-October is given in Table 5.5, with values ranging between 34.3-62.0%.

Comparison of observed and simulated number of culled cubs per mother

Figure 5.2 shows the observed monthly mean number of culled cubs per mother against the simulated values, assuming an RBCT cub trappability of 60%, 70%, 80%, 90% and 100%

respectively. All comparisons between observed and simulated values for the number of culled cubs per mother assumed a normal distribution for the observed values.

The mean number of culled cubs per mother observed during May was significantly higher than expected under the assumption of an RBCT cub trappability of either 60% ($p < 0.001$) or 70% ($p = 0.038$), and under no conditions were there significantly fewer culled cubs per mother than expected during May. Similar results were also found for June and July. The mean number of culled cubs per mother observed during June was significantly higher than expected under the assumption of an RBCT cub trappability of 60% ($p = 0.001$), 70% ($p = 0.008$) or 80% ($p = 0.045$), and under no conditions were there significantly fewer culled cubs than expected. For July there were significantly more culled cubs per more only under the assumption of an RBCT cub trappability of 60% ($p = 0.024$), and as for May and June there were no conditions under which there were significantly fewer culled cubs per mother. Simulated values under all RBCT cub trappability assumptions for August-October inclusive were within the 95% confidence intervals of the observed number of culled cubs per mother.

Table 5.3: The observed mean number of culled cubs per mother (with 95% confidence interval) and the expected mean number of culled cubs per mother generated by the simulations (assuming RBCT cub trappabilities of 60-100%) for May-October inclusive.

	May	**Jun**	**July**	**Aug**	**Sep**	**Oct**
Observed	1.573 (1.446-1.700)	1.401 (1.274-1.527)	1.309 (1.195-1.422)	1.437 (1.115-1.759)	1.237 (1.086-1.388)	1.222 (1.092-1.352)
Simulated (RBCT cub trappability = 60%)	1.366	1.193	1.18	1.186	1.15	1.17
Simulated (RBCT cub trappability = 70%)	1.44	1.231	1.217	1.22	1.181	1.195
Simulated (RBCT cub trappability = 80%)	1.518	1.273	1.248	1.262	1.211	1.225
Simulated (RBCT cub trappability = 90%)	1.601	1.309	1.282	1.296	1.239	1.257
Simulated (RBCT cub trappability = 100%)	1.691	1.348	1.321	1.335	1.266	1.293

Table 5.4: Cumulative monthly post-emergent cub survival estimates (%).

May	Jun	July	Aug	Sep	Oct
100.0 (100.0-100.0)	96.9 (71.9-99.7)	96.9 (71.9-99.7)	80.3 (61.2-91.3)	70.5 (54.6-82.6)	70.5 (54.6-82.6)

Table 5.5: Percentage of cubs with a qualifying MAP for May-October inclusive.

May	Jun	July	Aug	Sep	Oct
62.0	37.1	34.3	42.9	40.7	43.4

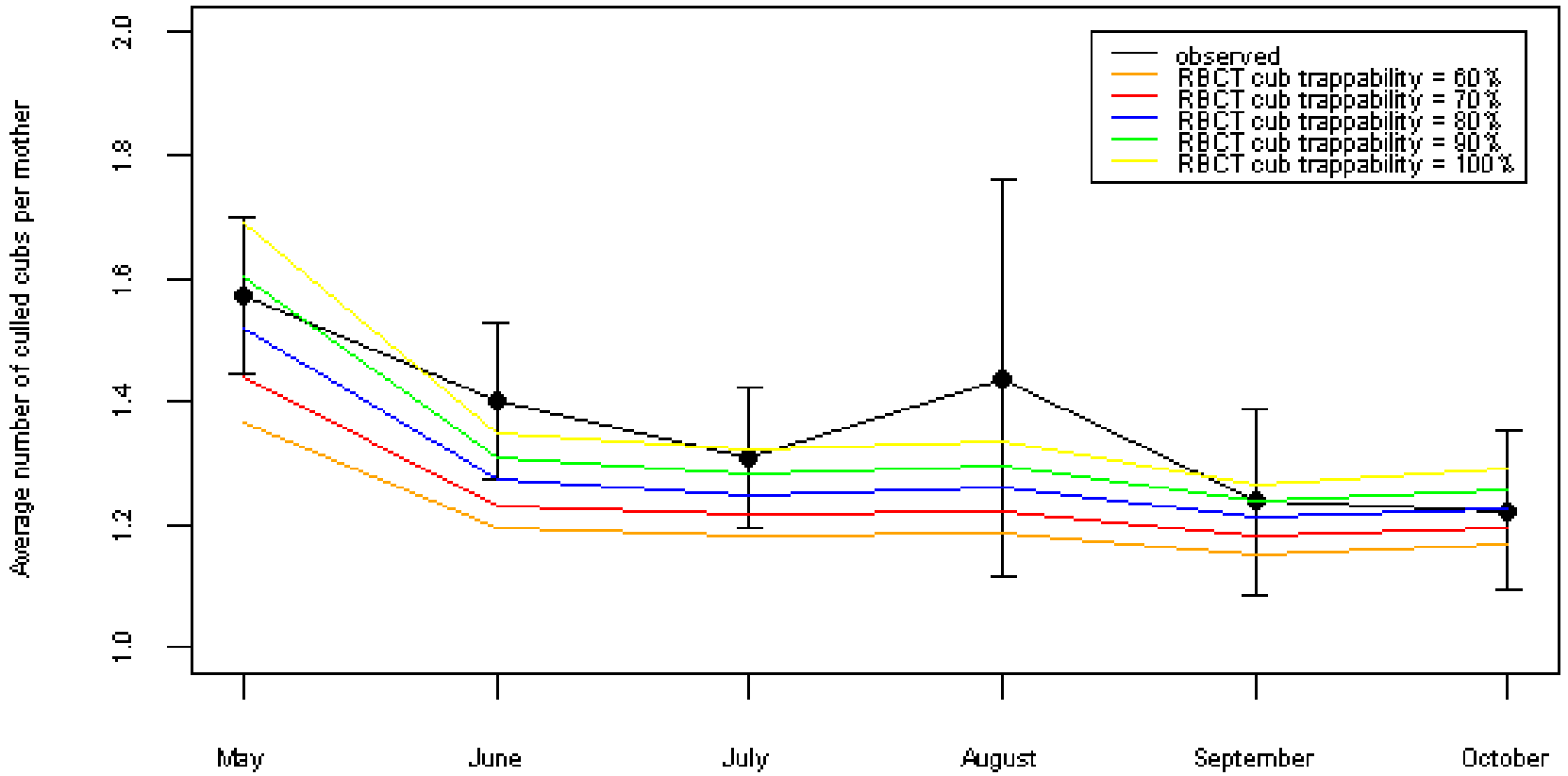


Figure 5.2: Graph showing the observed mean number of culled cubs per mother for each month (with 95% confidence interval error bars) against the expected values under the assumptions of 60%, 70%, 80%, 90% and 100% RBCT cub trappability.

Discussion

Of all cubs analysed, 66% (1,064 out of 1,611) were assigned a mother, and 66% of these cubs (702 out of 1,064) were assigned a mother with a qualifying MAP. Although this leaves less than half of the original 1,611 cubs, it is important that only maternal assignments of high MAP value are used, as maternal assignments of low MAP value are more likely to have the wrong adult female assigned as mother, and could consequently skew the mean number of culled cubs per mother values down if more truly cub-less adult females are assumed to be mothers. Applying an 80% cut-off reduced the number of mothers by 29% (712 to 503), with a similar reduction seen in other equivalent studies (Dugdale *et al.* (2007), a 39% reduction from 427 to 262).

The fact that every cull that had a mean of one culled cub per mother (the minimum value possible) had fewer than ten cubs with a qualifying MAP is not surprising, as having a small number of cubs is likely to produce a small mean value. The largest mean number of culled cubs per mother also came from culls with fewer than ten cubs with a qualifying MAP, with only A-4, B-3, D-4 and E-6 achieving mean values of 1.7 or higher. Such high values are likely to have been affected by stochastic variability, and thus are more likely to be observed in culls with few cubs. Seven culls had 20 or more qualifying cubs (A-2, D-2, E-1, F-1, F-2, G-2, H-2), and the mean number of culled cubs per mother for these culls ranged between 1.29-1.67.

The results of this work provide no evidence to suggest that culling using cage-traps in May misses dependent cubs of culled mothers, and so there is no evidence that culling badgers using this method in May would be inhumane on these grounds. Although Woodroffe *et al.* (2005a) estimated that approximately nine unweaned cubs per year were being missed through culling their mothers during the RBCT (using a method based on the lactation status of mothers), they also concluded that the closed season period used in the RBCT should not be altered. The significantly lower than observed values for simulations assuming low RBCT cub trappability (60% for May-July, 70% for May-June, 80% for June) might indicate that true RBCT cub trappability was high during these months. Furthermore, true RBCT cub trappability is likely to have varied between different months, as trappability of badgers is known to vary by season (Tuyttens *et al.*, 1999).

Comparing the estimated probability of a cub surviving until October of 70.5% (Table 5.4) with annual cub survival estimates elsewhere (other than from Macdonald and Newman (2002), which was used in deriving the estimates presented in this work), Rogers *et al.* (1997) estimated cub annual survival to be 53%. However, this estimate was combined with a pre-emergent mortality of 24%. Furthermore, Macdonald *et al.* (2009) estimated cub survival to be 67% (with standard error 3%). Therefore the value found in this work appears consistent with previous estimates.

The survival estimates from the Cormack-Jolly-Seber model were based only on cubs that were present in the Wytham Woods dataset, and thus did not take account of cubs that evaded capture (White and Burnham, 1999). Therefore it is possible that the survival estimates generated in this work are inflated. However, this was offset as best as possible by adjusting the Wytham Woods cub trappability value so that cub survival to October matched cub survival estimates from the same area and same years (Macdonald and Newman, 2002). An alternative statistical capture-recapture model is the Jolly-Seber model (Jolly, 1965, Pollock *et al.*, 1990, Schwarz and Arnason, 1996, Seber, 1965, Seber, 1982, Seber, 1986, Seber, 1992), which has the capability of estimating survival, trappability and population abundance. Although this may seem ideal for a dataset with unknown overall cub abundance (as with the Wytham Woods dataset presented here), a critical assumption of this model is that cage-trapped animals are as likely to be trapped as animals that are never trapped. In badgers, 'trap-happiness' and 'trap-shyness' is a well-documented heterogeneous characteristic (Byrne *et al.*, 2012, Tuyttens *et al.*, 1999) which would void this critical assumption of Jolly-Seber models. Previous work looking at UK badger populations have also found that Jolly-Seber models overestimate badger abundance (Rogers *et al.*, 1997).

An important caveat to the methodology presented in this work is that it is based on the assumption that there were not a high number of missed whole litters of culled mothers. Mothers of litters that were wholly missed would not have been identified in this work, and thus are not accounted for. However, analysis based on the lactation status of adult females culled during the RBCT proactive culls estimated that an average of just three whole litters per year were being missed (Woodroffe *et al.*, 2005a). Furthermore, Neal and Cheeseman (1996) estimated that 85% of litters are born by mid-March, and so under the assumption that cubs emerge approximately 8 weeks after birth (Neal and Cheeseman, 1996), it is therefore likely that the vast majority of litters have at least one post-emergent cub by May. However, further evidence based on more direct monitoring of young cubs would be required to ensure

a closed season period such as the one employed in the RBCT was sufficiently long to ensure a large number of cubs were not being missed.

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Chapter 6: Can disease-induced mortality suppress badger (*Meles meles*) social group size?

Abstract

European badgers (*Meles meles*) are an important wildlife source of *Mycobacterium bovis*, and understanding the population and disease dynamics of infected populations of badgers can provide key insights into managing the disease in both badgers and cattle. This chapter explores demographic and epidemiological processes which might explain a significant negative association observed between badger social group size and *M. bovis* prevalence, specifically investigating whether disease-induced mortality could be responsible for driving such a relationship. An individual-based stochastic simulation model was developed for 484 badger social groups connected on a hexagonal "honeycomb" grid. Processes captured by the model include births, deaths, dispersal, infection transmission and disease progression. Individuals within the model were classified by age, sex and infection status. Both density-dependent and frequency-dependent transmission assumptions were modelled, and the level of disease-induced mortality was varied. Models were initially simulated for 10 years, followed by logistic regression analyses of the model output variables for the first subsequent time step to characterise the level of association between social group size and prevalence.

All of the models investigated showed that as disease-induced mortality increased, the probability of observing a significant negative association between social group size and prevalence increased, and the probability of observing a significant positive association decreased. Disease-induced mortality appeared to have a stronger effect on the frequency-dependent transmission models, however both sets of models were qualitatively similar, particularly for higher levels of disease-induced mortality.

In the absence of disease-induced mortality there were a greater number of significant positive associations between social group size and prevalence than expected by chance. Over all the models simulated, an interpolated disease-induced mortality level of approximately 50% gave a 50% chance of observing a significant negative association between social group size and prevalence.

These results improve the understanding of the dynamics of badger populations in which the level of disease-induced mortality is suppressed, either naturally (due to a plentiful supply of resources) or artificially (through a vaccine that reduces the progression and severity of the disease, such as BCG), by understanding relationships between group size and *M. bovis* prevalence.

Introduction

Mathematical treatment of epidemiological dynamics has been studied for many years (Anderson and May, 1979, May and Anderson, 1979), and can be highly informative in both understanding the dynamics of infectious disease spread and persistence throughout various population structures (Eames and Keeling, 2002), as well as being able to model the impact of a disease intervention strategy such as a vaccination programme (Anderson and May, 1982, Anderson and May, 1985). Modelling is also a major field of ecology in its own right, with many important models focussing on population dynamics alone, for example in the study of predator-prey systems (Fryxell *et al.*, 2007, Holland and Hastings, 2008).

In this chapter I aim to construct a model that captures both ecological and epidemiological dynamics of *Mycobacterium bovis* in a badger (*Meles meles*) population. Firstly, I present an overview of existing models of *M. bovis*- infected badger populations, together with key results of these models. There has also been substantial work developing dynamical models of TB transmission based on the ecology and epidemiology of *M. bovis* in New Zealand possum populations. Although there are several differences between badger TB in England and possum TB in New Zealand, there are also many similarities between the two situations, in particular their shared status as being a wildlife host of *M. bovis* infection capable of transmitting bovine TB to cattle. I therefore also present a literature review of models that have been developed and used in this field, highlighting key insights from work on similar problems.

Badger TB models

There have been several mathematical models developed to aid in the understanding of the population dynamics of badgers in the UK, and how *M. bovis* infection amongst badgers affects these dynamics. The first of these, in what was one of the most extensive papers on the topic, was "Population dynamics of the badger (*Meles meles*) and the epidemiology of bovine tuberculosis (*Mycobacterium bovis*)" by Anderson and Trewhella (1985). The paper constructs numerous models, each considering different aspects of badger ecology, including fecundity, mortality, population age and sex structure, dispersal, density and abundance fluctuation, and the intrinsic growth rate (r, defined as the rate at which a population grows in the absence of external population-suppression forces). The paper begins by constructing models without *M. bovis* transmission, starting with the logistic model:

$$\frac{dN}{dt} = rN(1 - \frac{N}{K})$$

where $K = the\ carrying\ capacity,$

$N = population\ density,\ and$

$r = intrinsic\ growth\ rate.$

A fundamental assumption of this model is that the effective *per capita* growth rate, given by:

$$\hat{r}(N) = r(1 - \frac{N}{K})$$

(and is thus the co-efficient of *N* on the right-hand-side of the logistic model above) is linearly related to the population density *N*. Next they construct a variant of the logistic model that incorporates a density-dependent constraint, motivated by the relaxation of the assumption that the net growth rate, $\hat{r}(N)$, and overall population density, *N*, change in a linear fashion as the population density approaches the carrying capacity. This is given by:

$$\frac{dN}{dt} = rN[1 - \left(\frac{N}{K}\right)^{c}]$$

where $c = density\text{-}dependent\ constraint\ (in\ terms\ of\ r\ and\ K).$

Similarly, the co-efficient of *N* on the right-hand-side of this equation gives us the new definition of the effective *per capita* growth rate:

$$\hat{r}(N) = r[1 - \left(\frac{N}{K}\right)^{c}]$$

Age-structured models are considered next in the paper, the first of which is designed to improve the modelling of fecundity as a seasonal (discrete) process, and so is given by a difference equation model that includes annual fecundity and mortality rates, a density-dependent fecundity function, and age classes. Density equilibrium is again derived, and extensive stability analysis shows that various behaviours of the system are possible.

This model formulation is followed by an age-structured continuous model, motivated by the more continuous nature of mortality (compared with fecundity, which can be more accurately modelled as a discrete process), and is given by a partial differential equation (PDE) of *N(a,*

t), where *a* is the age of the badger and *t* is time. Analytical treatment of this model is limited, and the need for more data to validate the model is expressed by the authors.

The second modelling chapter of this paper attempts to incorporate disease transmission into the above models above, beginning with a simple, deterministic susceptible-exposed-infectious (SEI) model. This model aims to describe birth, baseline and disease-induced death, and pathogen transmission events, described by a system of ordinary differential equations (ODEs). The basic reproduction number R_0, defined as the mean number of secondary cases consequential of a primary case in a completely susceptible population, is derived from the model; however, the authors make it clear that a severe lack of epidemiological data makes their parameter estimates "at best crude estimates, and at worst, simple guesses". Following on from this model, they construct similar models that investigate the dynamics of the following: pseudo-vertical transmission (the increased risk of a cub becoming infected if it has an infected mother, caused by close mother-cub contact), a persistent source of infection on a pasture (local density of live bacilli), carriers (badgers that show no clinical evidence of infection, but are excreting bacilli and so are infectious), inactive cases (infected badgers temporarily or permanently non-infectious), seasonality, and the effect of stochastic fluctuations. Age-structured models were also included in the form of PDEs, however analytical progress was again limited by the complexity of the equations. It was shown, amongst other results, that pseudo-vertical transmission could feasibly play a key role in the persistence of the infection within badger populations, and that stochasticity could perpetuate any oscillatory behaviour of the system (in contrast, oscillatory behaviour was predicted as being 'damped' in the deterministic models).

The models used in Anderson and Trewhella (1985) also formed the basis of the models in "Modeling Bovine Tuberculosis in Badgers" by Bentil and Murray (1993); however a key difference in the models presented in this paper was the inclusion of an 'immune' class of infection. Bentil and Murray (1993) also focused on using a Logical Parameter Search (LPS) method to estimate parameter values for the models, in which parameter values are chosen to satisfy mathematical stability criteria relating of the existence of periodic solutions (Bentil and Murray, 1991). These results were then compared to similar ecological and epidemiological values from relevant literature. Another key assumption in this paper was that the latent period of infection (the time spent in the 'exposed' class) was long when compared with the infectious period; the authors justified the inclusion of this term by saying this effect is needed to predict the incidence and prevalence patterns observed in Anderson

and Trewhella (1985). Bentil and Murray produced a non-age-structured model (described mathematically using ODEs) and an age-structured model (using PDEs), and the methods and results were of a similar nature to Anderson and Trewhella (1985). Their stability analyses gave parameter thresholds about which the system behaviour switched between damped oscillatory and cyclic behaviour.

Anderson and Trewhella's model was further analysed in "The Effects of Stochasticity and Seasonality on Model Dynamics: Bovine Tuberculosis in Badgers" (Ruxton, 1996), and follows on from a conjecture made in Bentil and Murray (1993) that stochasticity and seasonality may "continually pump the otherwise damped oscillations, locking the system into sustained cycles". However, analyses of the models with these modifications did not show that this was the case, and was considered in contrast to other diseases (Aron and Schwartz, 1984, Dietz, 1976, Schaffer, 1985), including childhood diseases (Rand and Wilson, 1991).

Simple models have also been used to compare fertility and lethal control strategies, with particular emphasis on social perturbation (Swinton *et al.*, 1997), as well as constructing simple models incorporating cattle in transmission (Cox *et al.*, 2005). The Swinton *et al.* (1997) model used data from Woodchester Park, Gloucestershire, UK (Delahay *et al.*, 2000, Rogers *et al.*, 1998, Swinton *et al.*, 1997, Tuyttens *et al.*, 1999) to compare these two control strategies, leaving open the possibility that lethal control could increase the spread of infection. Cox *et al.* (2005) found that when including cattle into the model, the estimated net reproduction number (which differs from the basic reproduction number in that not all individuals are assumed to be susceptible) of the epidemic ranged between 1.02-1.11, however the authors mentioned that more data were required for a more accurate assessment.

The models mentioned in the above papers have been heavily mathematical, and have often concentrated on obtaining analytical solutions to the model equations. This approach is useful for understanding the underlying principles of the ecological dynamics of badger populations, in particular for determining factors relating to: the persistence of *M. bovis* infection in badger populations, any cyclicity in disease prevalence or subpopulations, and stability analyses after a natural or controlled population suppression. However, these models were generally non-spatial, and this leaves them unable to account for the spatial heterogeneity of the host population, as well as any variations in the structure of the host habitat. The absence of spatial structure is therefore potentially detrimental to the accuracy of a model's

predictions, and the need for spatial structure in these models was highlighted in Bentil and Murray (1993), and later by Graeme Ruxton: "it is suggested that one of the fundamental assumptions underlying compartment models makes them unsuitable for representing this problem, which must be addressed using spatially explicit models" (Ruxton, 1996). Since 1996 there have been many spatial simulation models developed, together with a new approach to tackling the problem of modelling the spread of *M. bovis* infection in badgers.

One of the first badger TB simulation models was published in the paper "Bovine tuberculosis in badger (*Meles meles*) populations in South West England: the use of a spatial stochastic simulation model to understand the dynamics of the disease" (White and Harris, 1995b). The spatial framework for this model was a 10 × 10 cell grid, with each cell representing a badger social group. There were also 44 extra cells surrounding the grid (i.e. a boundary ring two cells deep) that allowed edge effects to be ignored. As in Anderson and Trewhella (1985) an SEI model was used, but now probabilities were used to represent movement between infection classes (instead of continuous rates), and these were applied stochastically at each three-month time step. Three age classes were also used: cub (less than one year of age), yearling (1-2 years of age) and adult; each badger was also assigned a gender. The processes applied at each time step included baseline and disease-induced mortality, dispersal and colonisation (dispersal to empty territories), transition between non-diseased and diseased states, and inter- and intragroup disease transmission. Births and maturity into a new age class processes (for cubs and yearlings) occurred every fourth time step (i.e., annually). The model allowed each social group to interact with all four non-diagonally adjacent social groups (through dispersal and intergroup transmission). Analyses of White & Harris' model suggested that *M. bovis* infection may persist within badger populations for long periods of time even when the disease-free equilibrium group size was small (approximately 4 adults and yearlings); for an endemic situation the equilibrium group size was slightly larger (approximately 6 adults and yearlings). In this endemic situation, a mean prevalence of 11-22% was predicted.

White and Harris (1995b) was coupled with a follow up paper, entitled "Bovine tuberculosis in badger (*Meles meles*) populations in South West England: an assessment of past, present and possible future control strategies using simulation modelling" (White and Harris, 1995a). Here, various realistic measures of disease control were implemented into the previous paper's model, comparing different methods of culling to a (currently undeveloped) oral vaccine. Variations of both methods were considered as practical solutions to controlling the

spread of *M. bovis* infection in badgers, including gassing, the clean ring strategy, the interim strategy, single and repeated vaccinations, and live test methods (as outlined in Chapter 2). Of these, the analyses revealed that gassing and the clean ring strategy were optimal for control of localised infection. However, White & Harris concluded that the only strategy likely to eliminate the disease was repeated vaccinations over a long period of time in proactive control operations. The same model was then used again in a later paper to test whether fertility control would be an effective measure (White *et al.*, 1997), concluding that complete disease elimination was near impossible under the assumed model conditions.

Another similar spatially-explicit model was developed around the same time (Smith *et al.*, 1995), again using stochastic simulations to construct six models with different inter-group structures, the inclusion of pseudo-vertical transmission, and different levels of susceptibility to infection. Three of the models gave a prevalence that was in accordance with data obtained from the study at Woodchester Park, Gloucestershire, UK (Delahay *et al.*, 2000, Rogers *et al.*, 1998, Swinton *et al.*, 1997, Tuyttens *et al.*, 1999), and a key difference to previous models was the inclusion of a 'super-infectious' class, such that any badger in this class had a greater probability of transmitting infection to susceptible individuals than normally infected badgers. Badgers in this super-infectious class also had an extra level of mortality known as disease-induced mortality (additional to a baseline mortality subjected to all individuals). Further work using these models was presented in a follow-up paper (Smith *et al.*, 1997), looking at how effective different levels of culling on six badger social groups were as control strategies. The analyses suggested that if only infected animals were killed then it could be possible to eliminate the disease, however this was under the assumption of a perfect live test. The paper also suggested that killing lactating sows might have no significant effects unless the strategy was used over a much larger scale. Another key assumption in the model was that there was no change in behaviour in the remaining badgers after a nearby removal of infected badgers.

Until this point in time, models had focussed purely on badger TB ecology and epidemiology, and had not included cattle explicitly. The first model to include a cattle element was in "A model of bovine tuberculosis in the badger *Meles meles*: the inclusion of cattle and the use of a live test" (Smith *et al.*, 2001b), in which an extension of the Smith *et al.* (1997) model was developed, predicting that the inclusion of transmission from badgers to cattle would lead to herd breakdowns being correlated to *M. bovis* infection in badgers and badger population density. The assumption of whether or not pseudo-vertical transmission was present was also

varied in the models of Smith *et al.* (2001b): either all cubs of an infected mother were infected, or any effect of pseudo-vertical transmission was ignored. The time step within these models decreased from one year to six months, in order to account for the rapid onset of TB in some badgers. Another innovative element of Smith *et al.* (2001b) was that it was the first to model gender differences in their respective probabilities of becoming super-excretors (Wilkinson *et al.*, 2000). The results and analyses of this paper were used to examine various control strategies in a follow-up paper (Smith *et al.*, 2001a), suggesting that of the historic culling methods, gassing and clean-ring strategies were expected to have been the most effective at reducing infection prevalence in badgers and cattle herd breakdowns.

Other approaches to modelling spatial structure have included models that have preserved the spatial arrangement of studied badger social groups at Woodchester Park (Shirley *et al.*, 2003) rather than grids. Shirley *et al.* (2003) also performed an extensive sensitivity analysis, exposing key parameters that affected the medium-term (20 years) badger population size, and that population size combined with rates of the infection leaving social groups were most influential on the prevalence of TB.

Wilkinson *et al.* (2004) analysed various vaccination strategies within a model framework, using a modified version of the Smith *et al.* (2001b) model. They found that mass vaccination with low individual probability of success (80% of social groups vaccinated with 10% of vaccinated individuals successfully immunised) was more effective than vaccinating fewer social groups (10%) with a high probability of successfully immunising vaccinated individuals (80%), however mixed control strategies were not considered. In particular, culling-induced badger perturbation had not been taken into account, and it was around this time that the importance of the effect of perturbation began to come to light (Donnelly *et al.*, 2003). Further cost-benefit analyses were later published (Wilkinson *et al.*, 2009) using a model based again on the Smith *et al.* (2001b) model. Wilkinson *et al.* (2009) predicted that if the effect of perturbation was restricted (for example, due to natural barriers) then although it would be possible for badger culling to reduce the number of herd breakdowns, it would not be cost-effective.

More recent modelling work has focused on using existing models to investigate new aspects of the ecology and epidemiology of TB in badgers since the completion of the Randomised Badger Culling Trial (RBCT). In light of gaining a clearer understanding of the impacts social perturbation has on the spread and persistence of bovine TB since the RBCT (Jenkins

et al., 2007), Smith *et al.* (2012) adapted a model from earlier work (Smith *et al.*, 2001b) in order to compare the following bovine TB management strategies: large-scale proactive culling, badger vaccination, a combination of the two, and no action. They found that a combination of both proactive culling and vaccination on surrounding lands prevented the largest number of herd breakdowns, however their results were highly sensitive to low rates of land access, low culling efficacy, and the early termination of a culling strategy.

There has also been recent work analysing the White and Harris (1995b) model to understand the impact of external sources of infection, such as other species or environmental sources (Hardstaff *et al.*, 2012). Hardstaff *et al.* (2012) found that external infection sources are far more important if the threshold equilibrium group size is below 6 adults rather than above, and concluded that such sources of infection play a significant role in lower density populations. Further investigation into how vaccination strategies may be affected by external infection sources was also carried out recently (Hardstaff *et al.*, 2013), concluding that if such infection sources persist, then a vaccination strategy would be essential to reduce the prevalence levels of TB in badgers.

As well as mathematical and simulation models found in the literature discussed, the statistical multistate models developed in Graham *et al.* (2013) give evidence of some of the key ecological and epidemiological dynamics in badger TB, and could be used to further investigate the behaviour of such systems.

Possum TB models

There has also been much research into the development of models for the problem of bovine TB in cattle and possums in New Zealand, and so an overview of these models may prove to be useful to inform efforts to model the spread of the disease in the UK. The first possum TB models were developed by Barlow (1991b), in which he produced three deterministic SEI models: one with homogeneous mixing, the second with disease aggregation, and the third with age structure. Disease aggregation was achieved in the model by having diseased and non-diseased 'patches' that were governed by different dynamics. The dynamics were described using differential equations, and here mortality and pseudo-vertical transmission was assumed to be density-dependent. Barlow's model estimated the intrinsic rate of increase of possums was 0.2-0.3 $year^{-1}$, and that mortality was more dependent on density than recruitment was. It was also predicted that possum populations would tend to have a rapid time to recovery to equilibrium after a disturbance (e.g. culling), as well as low disease

prevalence. This was in contrast to work on general animal disease dynamics up to that point (Anderson and May, 1979, Anderson and Trewhella, 1985, May and Anderson, 1979), and was explained by the inclusion of disease aggregation. Barlow published a follow-up paper, using the disease-aggregated model to derive parameter thresholds for the likely effectiveness of culling, sterilisation and vaccination as methods of disease control (Barlow, 1991a). He predicted that culling and sterilising could be successful in achieving disease control if applied to a high enough proportion of the population, as too could vaccination. Cost-effectiveness analysis estimated that dropping 1080 poisoned baits over areas of at least 50km^2 every six years would be optimal with respect to possum population reduction, as well as reduced incidence in nearby cattle, and was subsequently used as a control measure. Although analysis of the disease-aggregated model was used for control policy, age-structure has also been seen to play an important role in economic application (Efford, 1991).

Two years later Barlow developed another model (Barlow, 1993), this time including an element of stochasticity into an originally deterministic model in order to better capture the randomness of dispersal and intergroup transmission events. This was done by modelling 'within-site' dynamics as continuous, deterministic events on patches, and modelling stochastic events between sites using a 20 × 50 km grid, with 1km^2 squares. On the border of regions that were either endemic with TB in possums, or had eliminated possums, the use of 'buffers' were implemented with the idea of preventing diseased possums from leaving endemic areas or entering possum-free areas. Analysis of this control measure predicted that buffers needed to be at least 12km wide in order to prevent juvenile possums from dispersing, and so to make the control measures as cost-effective as possible. Other methods of incorporating stochasticity into originally deterministic models have been attempted (Roberts and Saha, 1999), in which stochastic environmental variability was modelled as Gaussian white noise about the disease transmission rate.

Further investigation into the potential application of culling and vaccination as control methods came from "The dynamics of bovine tuberculosis in possum populations, and its elimination or control by culling or vaccination" (Roberts, 1996), in which a standard susceptible-infectious (SI) model was modified with an additional 'vaccinated' class. The model suggested that if the TB-free carrying capacity could be reduced by 57%, annual rates of culling (9.2%) or vaccinating (15%) would be required to achieve disease elimination, although the author also found the results to be highly sensitive to changes in parameter values.

Another important aspect of modelling the spread of diseases, as mentioned previously, was the inclusion of spatial heterogeneity. This has been demonstrated in metapopulation models (for example Fulford *et al.*, 2002) as well as spatially-explicit individual based models (Ramsey and Efford, 2010). Fulford *et al.* (2002) created a model framework incorporating distinct patches that were connected by dispersal paths for juveniles, and allowed for direct computation of the basic reproduction number, R_0. Ramsey and Efford (2010) examined the use of the oral vaccine bacillus Calmette-Guérin (BCG), as an alternative method to poisoning, due to public concern over the method of poisoning (Aldwell *et al.*, 2003, Buddle *et al.*, 2006). This was achieved via a grid-structure with 'home-range' centres represented mathematically by a Dirac δ-function, and concluded that if the BCG vaccine was combined with a single initial cull then this approach could be cost-effective through achieving local disease elimination in possums.

There has also been much work focusing on stability analyses of these types of models (Louie *et al.*, 1993, Roberts, 1992, Roberts and Kao, 1998). The model of Roberts (1992) was a generalised version of that found in Barlow (1991a), and assumed productivity, mortality and pseudo-vertical transmission were also density-dependent. Roberts also analysed three main methods of control (culling, sterilisation and vaccination), and found that to achieve elimination of *M. bovis* infection in possum populations, annual levels of culling, sterilisation or vaccination needed to be at 17.8%, 7.34% or 33.2% respectively. Roberts and Kao (1998) concentrated on birth pulses, and highlighted the importance between how fecundity and mortality should be modelled differently (i.e. discretely and continuously, respectively).

Other modelling work in this field include a simulation model of TB in cattle (Barlow *et al.*, 1997), in which analysis showed that a more sensitive diagnostic test in cattle would improve disease control in possum-free regions, however this would be much less effective in regions inhabited by possums. Roberts (1995) developed several intensity-based models, which were used to demonstrate that periodic system behaviour could not be obtained under such a model framework. Modelling work has also been conducted on the population control of possums, completely independent of any focus on TB (Barlow, 1994), reflecting possums' status as a non-native pest species in New Zealand, in contrast to badgers in the United Kingdom and the Republic of Ireland.

The link between social group size and *M. bovis* prevalence in badgers

Many epidemiological models have suggested that potentially higher contact rates in larger groups of individuals should result in higher levels of group infection (Anderson and May, 1979). However Woodroffe *et al.* (2009) found that *M. bovis* prevalence was consistently higher in smaller badger social groups and at lower population densities. Several mechanisms were hypothesised to explain this result, one of which was the possibility of a confounding variable in the form of immigrant badgers. Analysis of this showed that immigrants were highly likely to be infected, as is consistent with other studies (Pope *et al.*, 2007, Rogers *et al.*, 1997, Vicente *et al.*, 2007), however immigrants were not significantly associated with being members of larger social groups. The possible effect of relatedness was also investigated, however this was also not associated with either prevalence or social group size.

One hypothesis not concerning any confounding variable was that disease-induced mortality might be suppressing the size of infected social groups. As suggested in Woodroffe *et al.* (2009), demographic data and modelling would be required to ascertain whether such a mechanism exists, and if so what level of disease-induced mortality would be required to generate such dynamics. I therefore present a model attempting to capture the key ecological and epidemiological dynamics of *M. bovis*-infected badger populations, and use this model to investigate various conditions under which disease-induced mortality could create such an association between social group size and group prevalence.

Methods

Model structure

The model developed was a stochastic simulation model of badger population dynamics, with *M. bovis* infection present in the simulated population. Field data have shown that a reasonable mean number of neighbouring social groups is five (Smith *et al.*, 2001b), and so the model was simulated on a hexagonal "honeycomb" structure of 484 badger social groups (Figure 6.1), with each social group being connected to a maximum of six other social groups (Figure 6.2). Allowing for groups near the boundary of the hexagonal structure (with 2-5 neighbouring groups), as well as for the possibility of some groups becoming empty throughout the simulations, this was deemed to be a plausible network of badger social groups. Previously developed badger population simulation models have assumed each non-boundary group to be connected to four (White and Harris, 1995b) or eight (Smith *et al.*, 2001b) other neighbouring social groups. The total number of social groups in the structure was chosen to approximately equal the 501 social groups analysed in Woodroffe *et al.* (2009).

Model specification

A total of 11 age classes were tracked: cub (up to 1 year of age), yearling (up to 2 years of age) and nine adult classes (2, 3, 4, 5, 6, 7, 8, 9 and 10+ years of age), as badgers have been recorded as living longer than 10 years of age (male badgers up to 13 years, Dugdale, 2007). There were four states of *M. bovis* infection: susceptible, latent, infectious and super-infectious (Figure 6.3). The models were also sex-dependent. All assumed probability parameters (Tables 6.1 and 6.2) for transitions between different states were based on observed rates between states from the literature (Graham *et al.*, 2013, Macdonald *et al.*, 2008, Neal and Cheeseman, 1996, Woodroffe *et al.*, 1995).

Mortality

Mortality events were simulated stochastically every three months, and as in Graham *et al.* (2013) mortality was also assumed to be sex-dependent, but not age-dependent, with higher mortality probabilities in males (Graham *et al.*, 2013). The degree of disease-induced mortality (DIM), α, ranged between 0 and 1 at intervals of 0.2 (0 representing no DIM, 1 representing complete DIM).

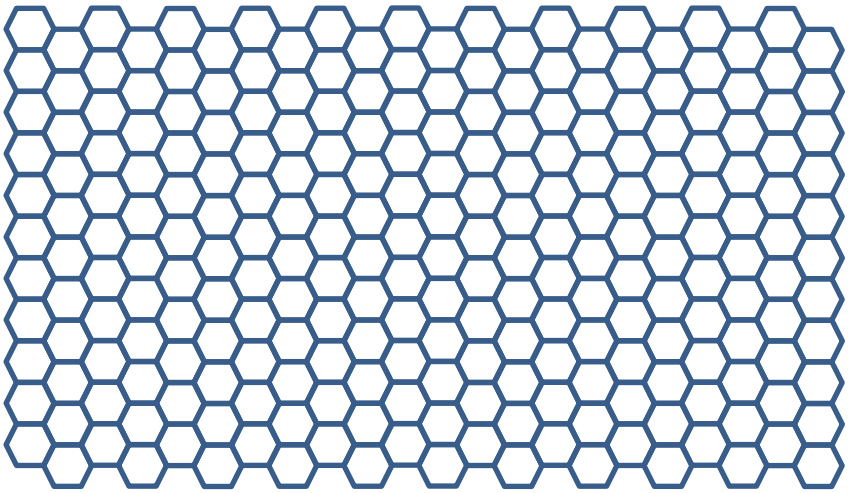


Figure 6.1: Structure of hexagonal social group network. Each hexagon represents a social group, with adjacent hexagons representing neighbouring social groups.

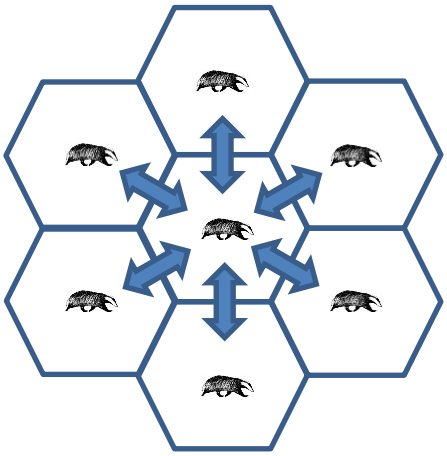


Figure 6.2: Dispersal around one social group, with each hexagon representing a social group.

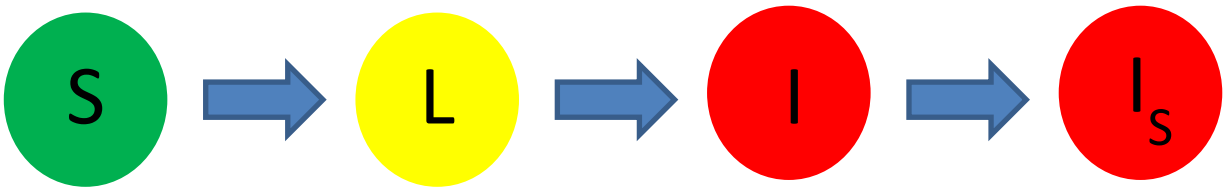


Figure 6.3: Susceptible (no infection), latent (infected but not infectious), infectious and super-infectious compartments of the simulation model.

Table 6.1 gives the general mortality probabilities in terms of the level of DIM. These were defined such that when $\alpha = 1$ (that is, under the assumption of complete DIM), mortality probabilities were equal to those estimated in Graham *et al.* (2013).

Fecundity

Woodroffe *et al.* (2005) found no more than three mothers for each badger social group analysed, and so up to a maximum of three adult females from each social group had the potential to successfully produce a litter of cubs each year in the model. Only individuals aged two and over were considered to be sexually mature (Ahnlund, 1980, Woodroffe and Macdonald, 1995), thus only adult females were able to breed in the model. This was modelled as a Bernoulli trial with probability 0.75 (this value stabilised the population and prevalence levels under the assumption of complete DIM, for which mortality values were assumed to be known from Graham *et al.* (2013)). Litter sizes were modelled as being post-emergent in order to avoid uncertainty in pre-emergent cub mortality rates, and recent studies have shown that post-emergent cub mortality is not significantly different to other age-defined mortalities (Graham *et al.*, 2013). A distribution for post-emergent litter sizes was taken form Neal and Cheeseman (1996), with the probability of a litter size of 1, 2, 3, 4 or 5 defined being 0.11, 0.52, 0.29, 0.06 and 0.02 respectively, resulting in a mean post-emergent litter size of 2.36. All post-emergent cubs were initially classed as being susceptible, as results from Chapter 4 showed no evidence for pseudo-vertical transmission, and so cubs are highly unlikely to be infected at their first emergence.

M. bovis transmission

Two assumptions regarding the nature of intra-group transmission of *M. bovis* were modelled:

1. Density-dependent transmission
   The probability of a susceptible individual becoming infected depended only on the *absolute number* of infectious and super-infectious individuals within the group:

$$Probability\ (Susceptible \rightarrow Latent) = 1 - (1 - t_d)^{(I + \tau_s I_s)}$$

2. Frequency-dependent transmission

   The probability of a susceptible individual becoming infected depended only on the *proportion* of infectious and super-infectious individuals within the group:

$$Probability\ (Susceptible \rightarrow Latent) = 1 - (1 - t_f)^{\frac{(I+\tau_S I_S)}{N}}$$

   where t_d and t_f are transmission parameters to be estimated;

   I = number of infectious group members;

   τ_s = relative infectiousness of super-infectious to infectious group members;

   I_s = number of super-infectious group members;

   N = total number of group members;

As in previous models of badger TB that have included a super-infectious compartment for disease status, super-infectious individuals are assumed to be twice as infectious to susceptibles as infectious individuals, and so τ_s was assumed to equal 2 (Smith *et al.*, 1997). Under the assumption of complete DIM ($\alpha = 1$), reasonable transmission parameters were estimated (in conjunction with estimating fecundity) to give population stability and prevalence levels of between 10 – 20% (14% found in Jenkins *et al.*, 2008). For all other degrees of DIM (α between 0 and 0.8), transmission parameters were estimated to again give stable population and prevalence levels. Values for t_d and t_f are shown in Table 6.2.

The relative importance of intergroup and intragroup infection transmission is difficult to estimate, however (Smith *et al.*, 2001b) gave an approximate estimate of the rate of intergroup infection transmission being just 6% of that of intragroup infection transmission. Therefore intergroup infection transmission was not modelled explicitly, and was instead modelled as a combination of intragroup infection transmission and dispersal. Patterns of *M. bovis* dynamics in badger populations show that new group infections are often associated with dispersal between social groups (Rogers *et al.*, 1998, Vicente *et al.*, 2007).

Ageing

As all badger cubs are born around the same time of year (the vast majority in February, Woodroffe *et al.*, 2005), all individuals transferred to the next older age class annually during

Table 6.1: Full model parameter table and sensitivity analysis values (with generalised mortality values in terms of the DIM α). All probabilities assumed to be over a three month period unless otherwise stated.

Parameter	Value	Values used in sensitivity analysis
Minimum number of resident adult females to prevent immigrant adult females joining	2	1, 3
Maximum number of mothers per social group	3	2, 4
Probability of latent female becoming infectious	0.047	0.0235, 0.0705
Probability of latent male becoming infectious	0.071	0.0355, 0.1065
Probability of infectious female becoming super-infectious	0.071	0.0355, 0.1065
Probability of infectious male becoming super-infectious	0.107	0.0535, 0.1605
(Baseline) probability of female dispersal	0.0013	0.00065, 0.00195
Probability of male dispersal probability	0.014	0.007, 0.021
Relative infectiousness of super-infectious to infectious	2	1, 3
Time increment (months)	3	1
Annual breeding probability	0.75	0.625, 0.875
Probability of susceptible female mortality event	0.074	0.037, 0.148
Probability of susceptible male mortality event	0.094	0.047, 0.188
Probability of latent female mortality event	0.074	0.037, 0.148
Probability of latent male mortality event	$0.094 \times (1.445)^{\alpha}$	$0.047 \times (1.445)^{\alpha}$, $0.188 \times (1.445)^{\alpha}$
Probability of infectious female mortality event	0.074	0.037, 0.148
Probability of infectious male mortality event	$0.094 \times (1.887)^{\alpha}$	$0.047 \times (1.887)^{\alpha}$, $0.188 \times (1.887)^{\alpha}$
Probability of super-infectious female mortality event	$0.074 \times (3.083)^{\alpha}$	$0.037 \times (3.083)^{\alpha}$, $0.148 \times (3.083)^{\alpha}$
Probability of super-infectious male mortality event	$0.094 \times (5.056)^{\alpha}$	$0.047 \times (5.056)^{\alpha}$, $0.188 \times (5.056)^{\alpha}$
t_d (density-dependent transmission parameter)	$t_d(\alpha)$	$t_d^-(\alpha)$, $t_d^+(\alpha)$
t_f (frequency-dependent transmission parameter)	$t_f(\alpha)$	$t_f^-(\alpha)$, $t_f^+(\alpha)$

Table 6.2: Transmission parameters under the assumption of density-dependent transmission (t_d) and frequency-dependent transmission (t_f), for each level of DIM α.

α	**$t_d(\alpha)$ [$t_d^-(\alpha)$, $t_d^+(\alpha)$]**	**$t_f(\alpha)$ [$t_f^-(\alpha)$, $t_f^+(\alpha)$]**
0	0.060 [0.030, 0.090]	0.450 [0.225, 0.675]
0.2	0.070 [0.035, 0.105]	0.600 [0.400, 0.800]
0.4	0.090 [0.045, 0.135]	0.700 [0.550, 0.850]
0.6	0.130 [0.065, 0.195]	0.850 [0.775, 0.925]
0.8	0.200 [0.100, 0.300]	0.900 [0.850, 0.950]
1	0.750 [0.625, 0.875]	0.950 [0.925, 0.975]

the same quarter that new cubs were born, except for individuals aged 10+ years, who remained in that age class.

Disease progression

Once an individual moved from the susceptible compartment to the latent disease compartment of the model, they underwent a Bernoulli trial at every subsequent three-month time step to determine whether they then moved into the infectious compartment. The associated probability for this process for females was 0.047, and for males was 0.071 (values taken from Graham *et al.*, 2013). Similarly, once an individual moved into the infectious compartment of the model, it underwent a Bernoulli trial at every subsequent three-month time step to determine whether it moved into the super-infectious compartment. The associated probability for this process for females was 0.071, and for males was 0.107 (again values are taken from Graham *et al.*, 2013).

Dispersal

All yearlings and adults were given a probability of moving to a neighbouring social group at each three-month time step (Figure 6.2), and cubs were assumed not to disperse (Christian, 1994, da Silva *et al.*, 1994). Woodroffe *et al.* (1995) found that adult females were more likely to disperse from larger groups containing a greater number of adult females, and also frequently dispersed in groups of 2-3 to take over neighbouring groups with only one adult female. Each adult female from a social group containing more than five adult females was therefore given the following probability of emigrating:

$$Probability(adult\ female\ emigrating | A_F > 5) = \frac{A_F - 5}{A_F}$$

where A_F = number of adult females in the social group.

The above probability was defined in order to make the expected number of adult female emigrants equal to $A_F - 5$, thus leaving the expected number of adult females in the group at five, in order to model within-group female-female competition. Emigrating adult females were assumed to be twice as likely to move to neighbouring social groups with no adult females compared to neighbouring groups with one adult female, and neighbouring groups with two or more adult females were avoided. If all neighbouring groups had two or more adult females then no adult females emigrated from that group. If there were five or fewer

adult females in a social group, then no density-dependent dispersal function was applied, and each adult female dispersed with a fixed probability of 0.0013 for each 3-month time step (value taken from Graham *et al.*, 2013). Yearling females also dispersed to a random neighbouring social group with a quarterly probability of 0.0013. Adult and yearling males dispersed to a random neighbouring social group with a greater quarterly probability (Macdonald *et al.*, 2008) of 0.0140.

Initial conditions

Woodroffe *et al.* (2009) found the mean observed group size based on adult badgers taken during the initial culls of the RBCT and assigned a unique badger social group to be 4.76. In order to estimate the mean number of adults assigned to each simulated social group in the model, estimates of adult badger capture efficacy during the initial culls of the RBCT (estimated to be 72.8% from Chapter 3), and of the percentage of these captured adult badgers that were assigned a unique social group (89%, Woodroffe *et al.*, 2009), were used on simulated populations to find an appropriate value for the true mean adult group size. A mean of seven adult badgers per social group (following a Poisson distribution) applied to 1000 simulated populations gave a mean non-empty adult group size of 4.60 (95% CI: 4.42-4.79), and was thus consistent with the value of 4.76 observed in Woodroffe *et al.* (2009). Furthermore, assuming a cub:adult population ratio structure of 27:73 (Rogers *et al.*, 1997), this gives a mean total group size (including cubs) of 9.59, which is also consistent with values found in other badger populations (Tuyttens *et al.*, 2000). These randomly assigned adults were uniformly picked from all adult age classes. Each adult also had a 20% probability of being infected, giving an initial population prevalence of approximately 20%. This level declined once cubs were subsequently included into the model; the resultant prevalence was between 10 and 20% (Jenkins *et al.*, 2008).

Model simulations

Models were simulated under six levels of DIM (α equal to 0, 0.2, 0.4, 0.6, 0.8, 1) and under both transmission assumptions, making a total of 12 models. Each model was simulated 200 times for 10 years (model values during the first 10 years were ignored in case they were affected by the initial conditions), and the values at the first three-month time step after the first 10 years were recorded. No further values were recorded.

Statistical analyses

For each simulation, logistic regression analysis was performed during the last three-month time step to analyse the effect of group size on the probability of *M. bovis* infection in adults. Only the infection status of adults were analysed, as prevalence patterns in younger badgers are distinctly different to patterns in adults (Woodroffe *et al.*, 2006). Three measures of group size were recorded for each regression analysis:

a) Total number of group individuals (adults, yearlings and cubs);
b) Number of adults and yearlings in the group;
c) Number of adults in the group.

As in Woodroffe *et al.* (2009), these prevalence models were fitted using generalised estimating equations (GEE) to account for repeated measures from the same social group, and were also adjusted for age and sex. Five adult age classes based on tooth wear were defined in the analyses of Woodroffe *et al.* (2009), and so to mirror this five new adult age classes were constructed for the statistical analyses: 2-3, 4-5, 6-7, 8-9, and 10+ years of age.

For each of the 12 simulation models, the p-value and direction of association between the probability of being infected with *M. bovis* and group size (all three definitions above) were recorded at the final three-month time step of each of the 200 simulations. The proportion of these p-values that were statistically significant (both in the positive and negative direction) was then calculated. In the context of the regression analyses, the proportion of significant p-values will be compared to the proportion of significant p-values that would be expected to occur through chance alone (i.e. under the assumption of no significant association).

Comparison simulations

Longer simulations were also run to ensure that the population dynamics of the comparatively shorter simulations outlined above were being captured, and could also be captured from 'perturbing' a stable system with complete DIM ($\alpha = 1$). For each of the twelve model structures, 20 simulations were performed for 200 years: the first 100 years was under the assumption of complete DIM, followed by perturbing the system to the DIM value of the model structure and running for another 100 years.

Sensitivity analysis

Further analyses were performed by adjusting assumed parameter values to assess whether any observed qualitative behaviour of the system was sensitive to any of these assumed parameter values. For each of the twelve model structures, 20 pairs of corresponding simulations were performed for each assumed parameter value (one simulation for a value either side of the original assumed parameter value). Tables 6.1 and 6.2 give the assumed and alternative pairs of parameter values used in the sensitivity analyses.

Results

Density-dependent transmission simulation models

The proportion of significant positive associations between prevalence and all group size definitions declined as the degree of DIM increased, whereas the proportion of significant negative associations between prevalence and group size increased as DIM increased (Figure 6.4). The dotted line in each panel of Figure 6.4 represents the proportion of significant positive and negative associations that would be expected by chance (2.5% each), showing that a greater number of significant positive associations between group size and prevalence than expected by chance occurred at DIM values of either 0.4 and lower (Figures 6.4(a) and (b)) or 0.2 and lower (Figure 6.4(c)), whereas a greater number of significant negative associations than expected by chance occurred at DIM values of 0.4 and above.

The points of intersection of the pairs of lines in Figure 6.4(a), (b) and (c) all lie within DIM values of 0.2-0.6, and this signifies the region of DIM in which the system is equally likely to give a significant positive or negative association between group size and prevalence. Corresponding proportions of significant positive and negative associations between all group size definitions and prevalence were significantly different for DIM values of 0.2 and 0.6 ($p < 0.0001$ for both).

Several trends can be observed between the different group size definitions. The DIM value at the point of intersection of the two lines on each graph decreased as younger members were excluded from the group size definition (moving left to right from Figure 6.4(a) to Figure 6.4(c)). In general, the proportion of significant positive associations decreased for corresponding DIM values as younger members were excluded from the group size definition; in contrast, the proportion of significant negative associations increased for corresponding DIM values as younger members were excluded.

The mean annual population over all 200 simulations for each density-dependent model for the first 10 years in Figure 6.6 shows population sizes increasing for lower levels of DIM. Similar increases in prevalence, mean non-zero group size and the proportion of non-empty social groups for lower levels of DIM are shown in Figures 6.8, 6.10 and 6.12 respectively.

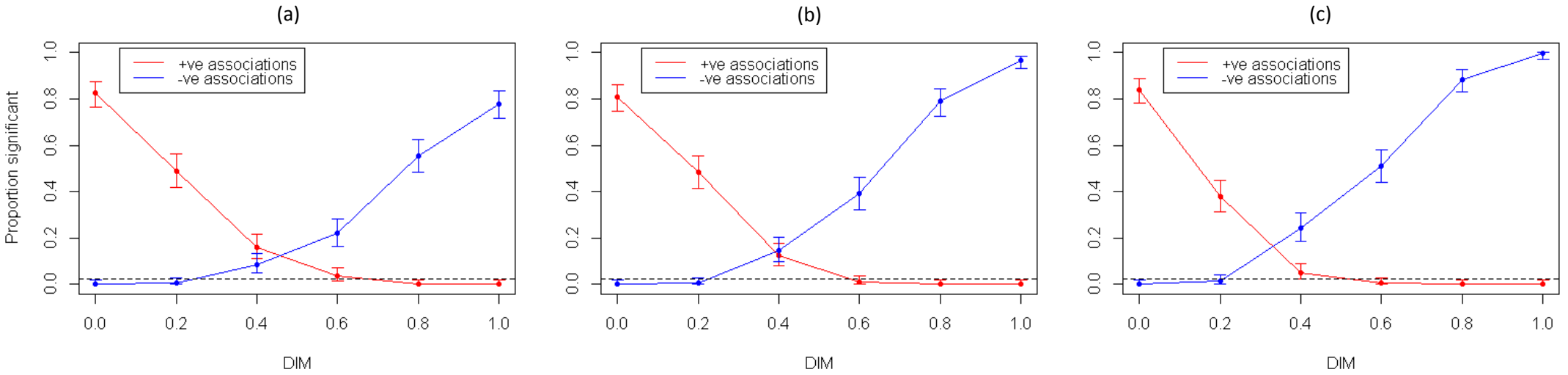


Figure 6.4: The proportion of significant positive (+ve) and negative (-ve) associations between prevalence and (a) total group size, (b) adult and yearling group size, (c) adult group size, under the assumption of density-dependent transmission, with 95% binomial confidence interval error bars.

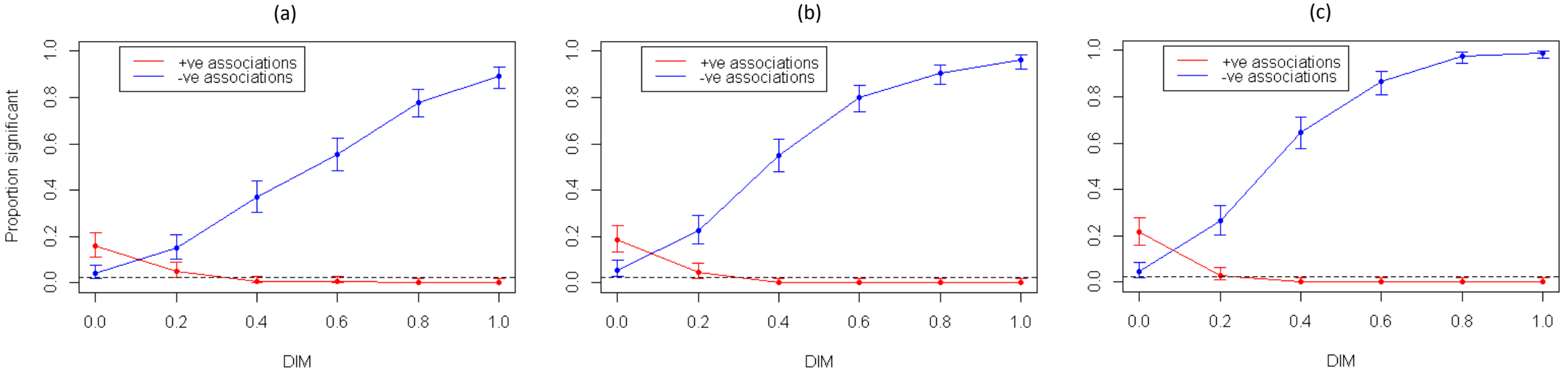


Figure 6.5: The proportion of significant positive (+ve) and negative (-ve) associations between prevalence and (a) total group size, (b) adult and yearling group size, (c) adult group size, under the assumption of frequency-dependent transmission, with 95% binomial confidence interval error bars.

Frequency-dependent transmission simulation models

Similar associations between prevalence, group size and DIM were observed for frequency-dependent transmission models, with the proportion of significant positive associations decreasing with increasing DIM, and the proportion of significant negative associations increasing with increasing DIM (Figure 6.5). In contrast to the density-dependent model results, a greater number of significant positive associations between group size and prevalence than expected by chance only occurred when DIM was 0, and a greater number of significant negative associations than expected by chance for either all DIM values (Figure 6.5(b)) or all non-zero DIM values (Figures 6.5(a) and (c)).

The point of intersection of the pairs of lines in Figure 6.5(a), (b) and (c) all lie within DIM values of 0-0.2, a region of DIM values that has no overlap with the corresponding region of 0.2-0.6 for the density-dependent models. Corresponding proportions of significant positive and negative associations between all group size definitions and prevalence are significantly different for a DIM value of 0 and 0.2 ($p < 0.0001$ for both).

As with the density-dependent transmission model results, the DIM value at the point of intersection of the two lines on each graph also decreased as younger members were excluded from the group size definition. There was no obvious trend in the change of the proportion of significant positive associations as younger members were excluded (moving left to right from Figure 6.5(a) to Figure 6.5(c)), however the proportion of significant negative associations increased as younger members were excluded.

Similar changes in the mean annual population observed in the density-dependent models were also seen for the 200 simulations for each frequency-dependent model for the first 10 years, with mean population sizes increasing for lower levels of DIM (Figure 6.7). Again, increases in prevalence, mean non-zero group size and the proportion of non-empty social groups for lower levels of DIM were observed, shown in Figures 6.9, 6.11 and 6.13 respectively.

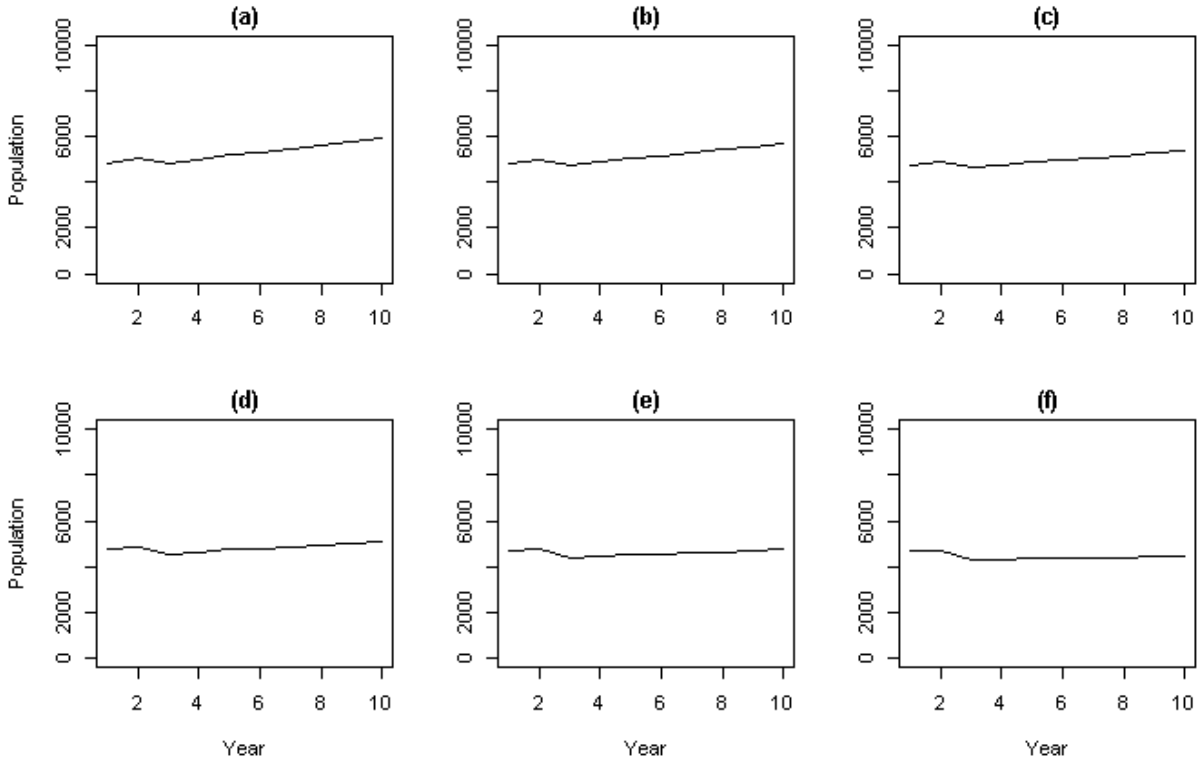


Figure 6.6: Mean annual population for density-dependent models with DIM at (a) 0, (b) 0.2, (c) 0.4, (d), 0.6, (e) 0.8, (f) 1.0.

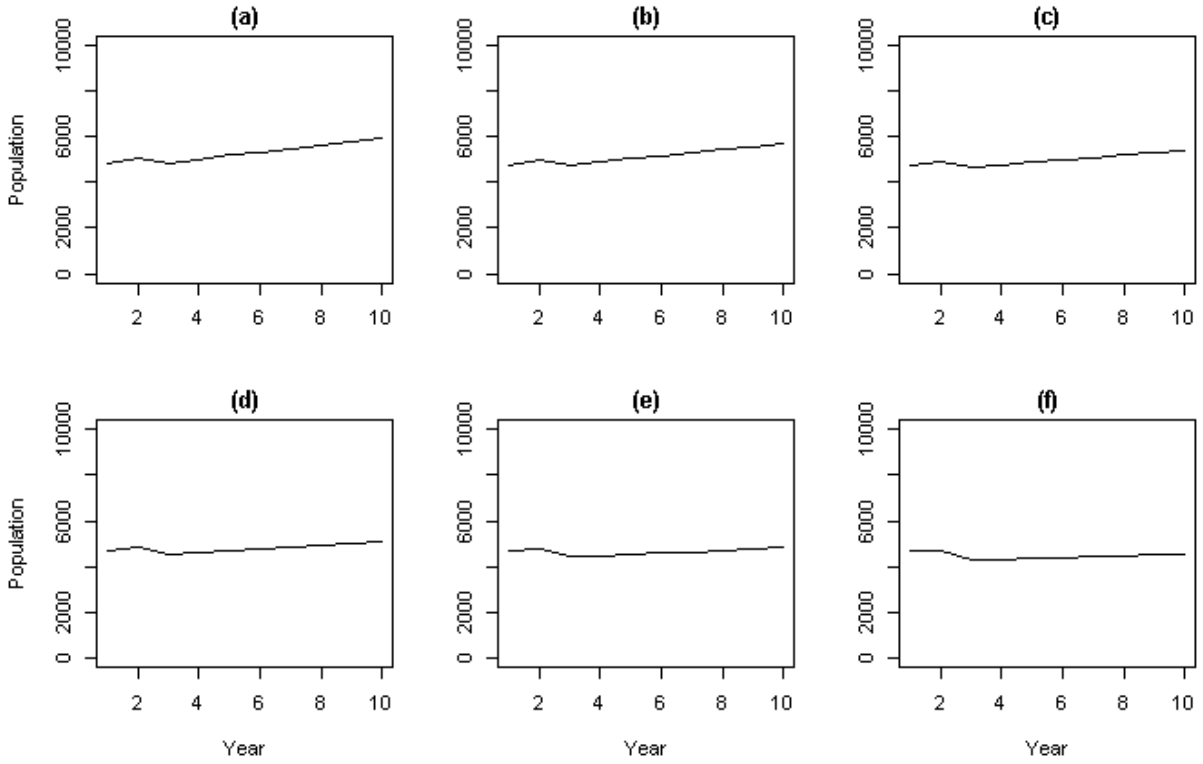


Figure 6.7: Mean annual population for frequency-dependent models with DIM at (a) 0, (b) 0.2, (c) 0.4, (d), 0.6, (e) 0.8, (f) 1.0.

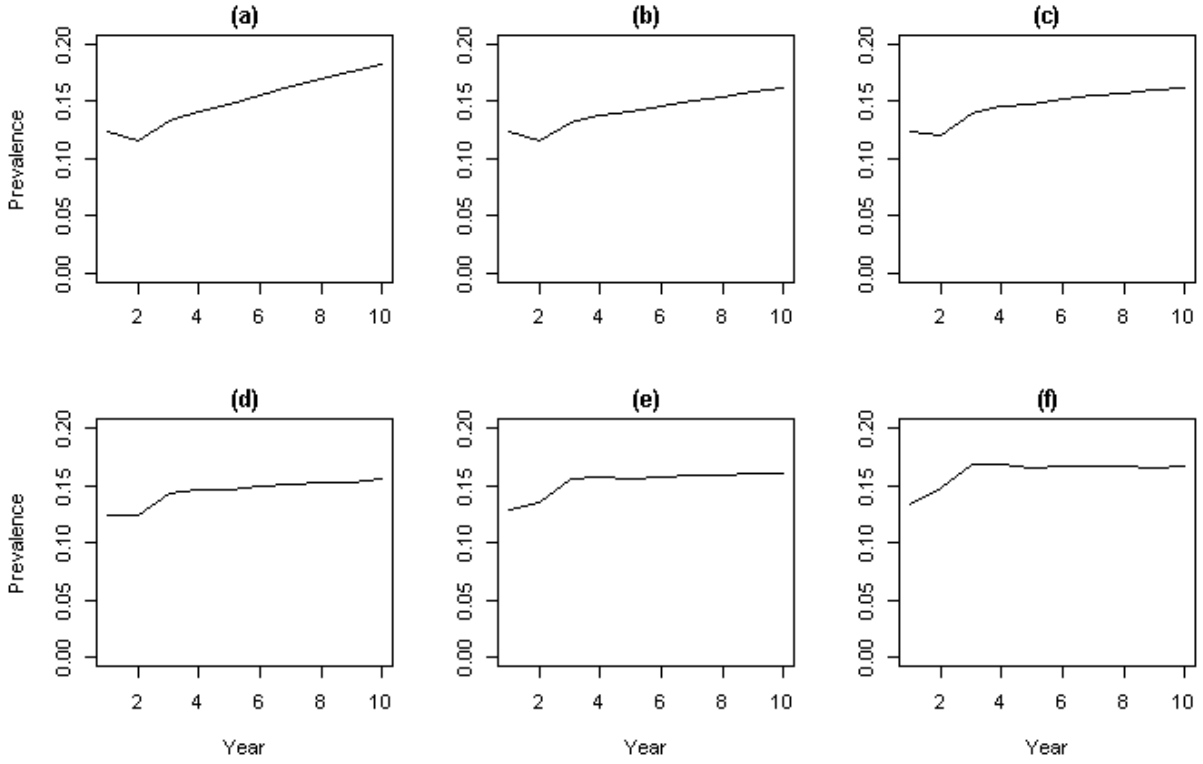


Figure 6.8: Mean annual prevalence for density-dependent models with DIM at (a) 0, (b) 0.2, (c) 0.4, (d), 0.6, (e) 0.8, (f) 1.0.

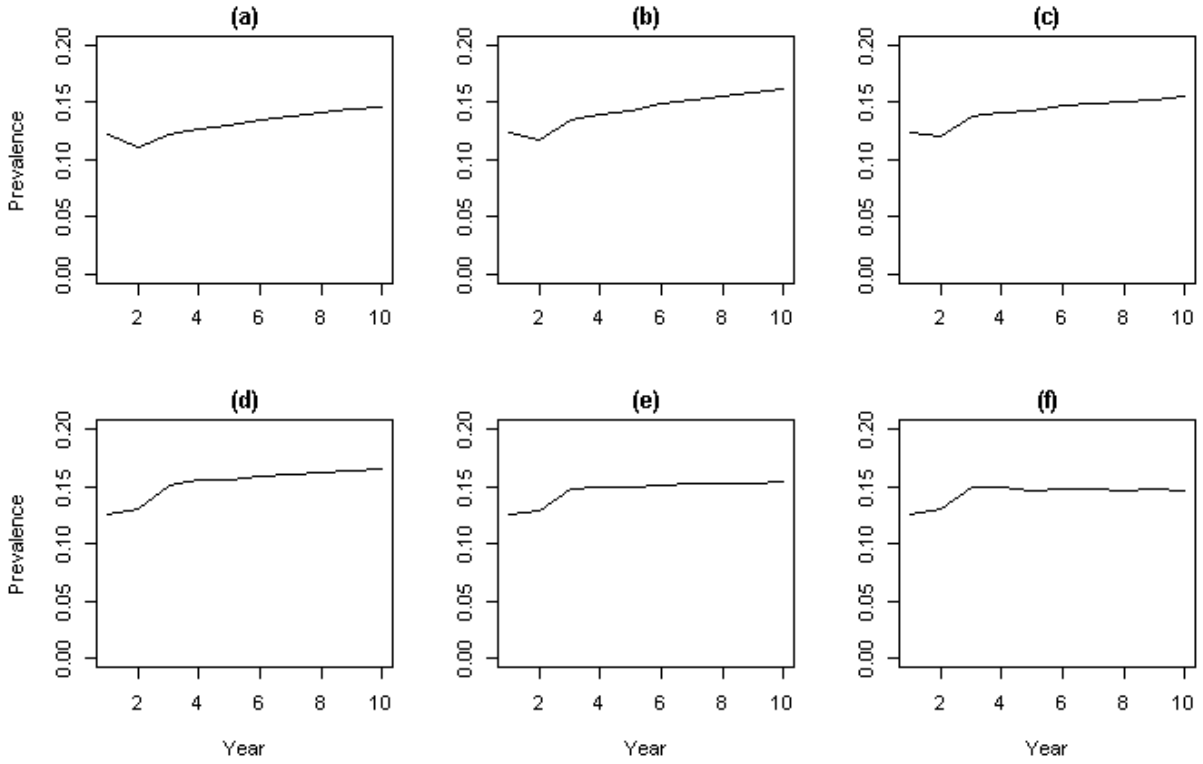


Figure 6.9: Mean annual prevalence for frequency-dependent models with DIM at (a) 0, (b) 0.2, (c) 0.4, (d), 0.6, (e) 0.8, (f) 1.0.

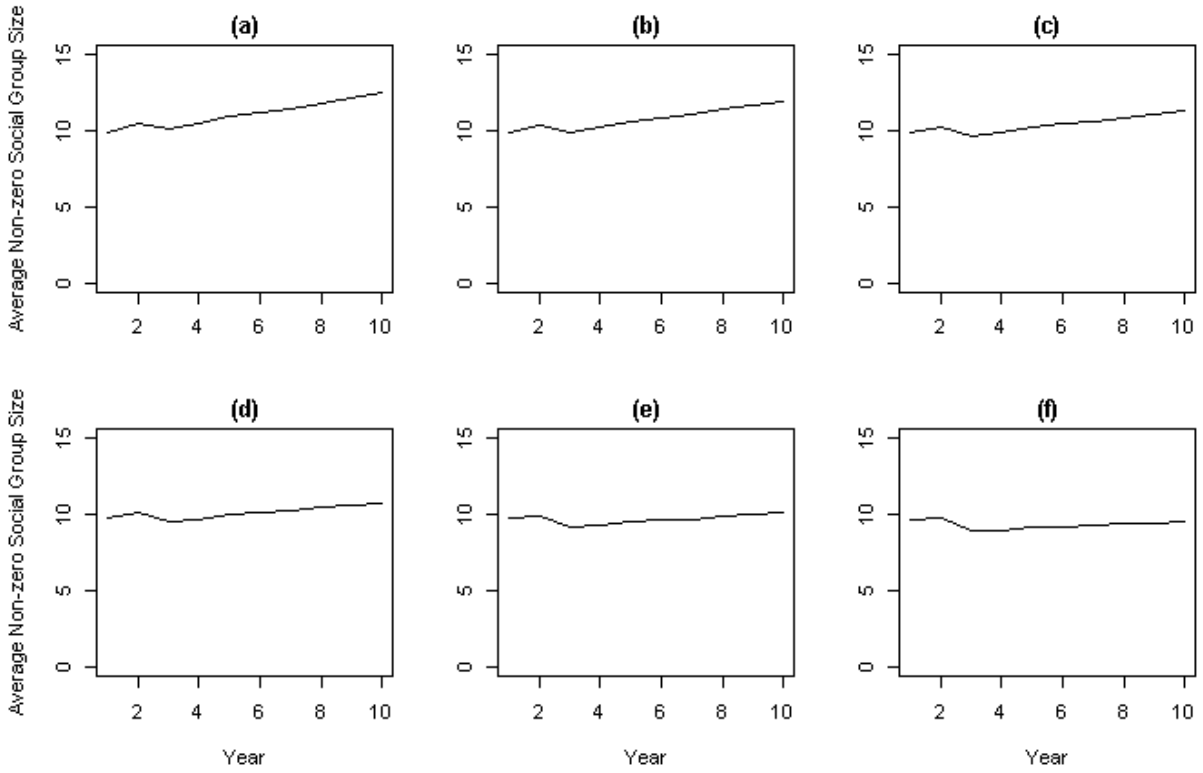


Figure 6.10: Mean annual non-zero social group size for density-dependent models with DIM at (a) 0, (b) 0.2, (c) 0.4, (d), 0.6, (e) 0.8, (f) 1.0.

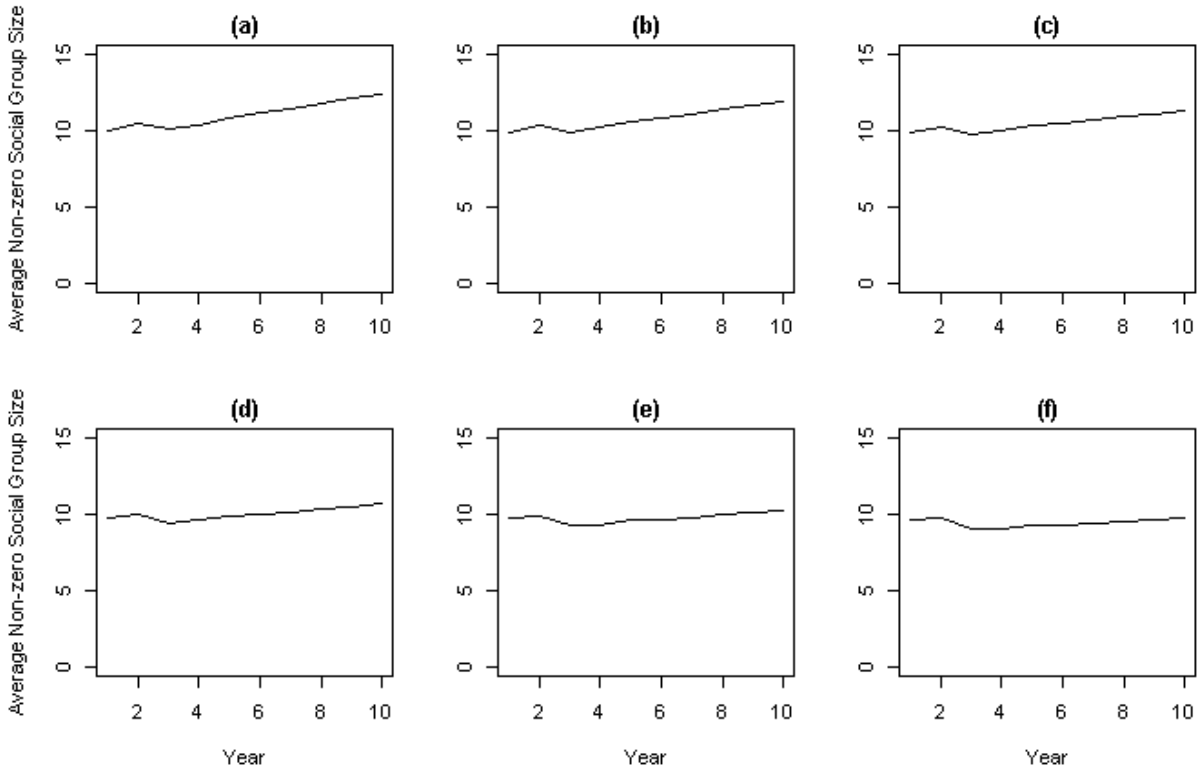


Figure 6.11: Mean annual non-zero group size for frequency-dependent models with DIM at (a) 0, (b) 0.2, (c) 0.4, (d), 0.6, (e) 0.8, (f) 1.0.

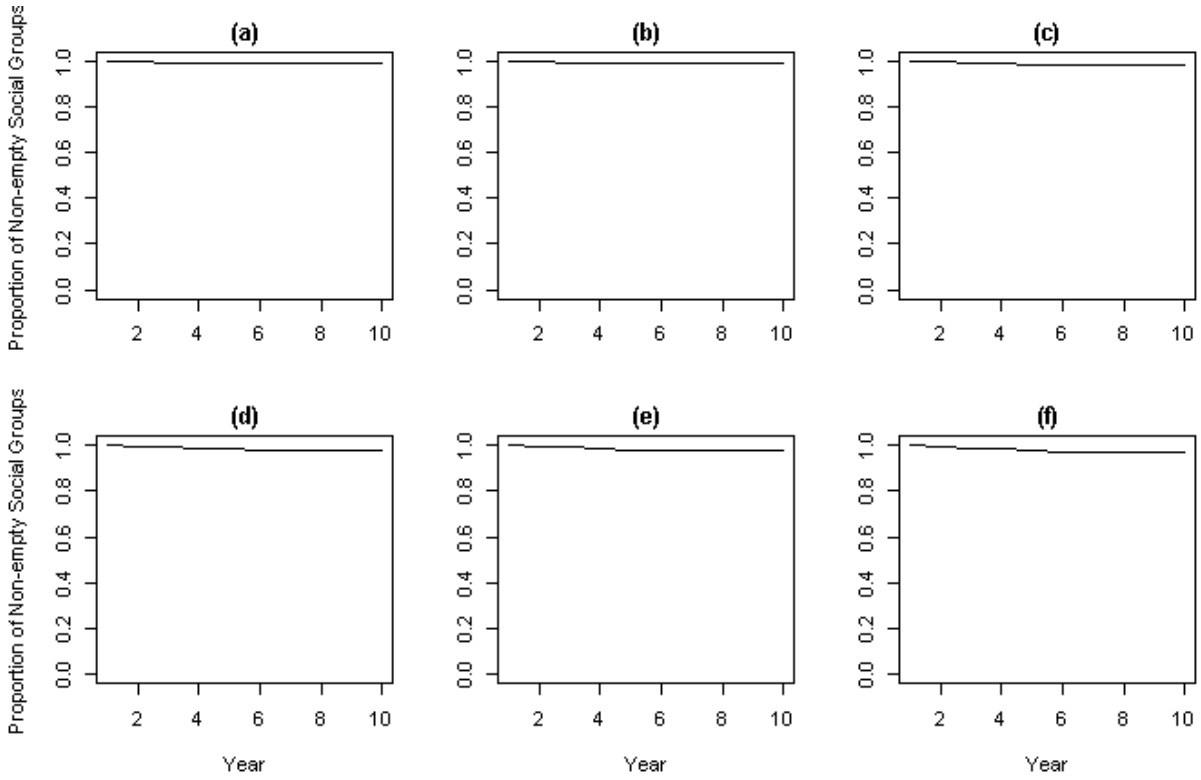


Figure 6.12: Mean annual proportion of non-empty social groups for density-dependent models with DIM at (a) 0, (b) 0.2, (c) 0.4, (d), 0.6, (e) 0.8, (f) 1.0.

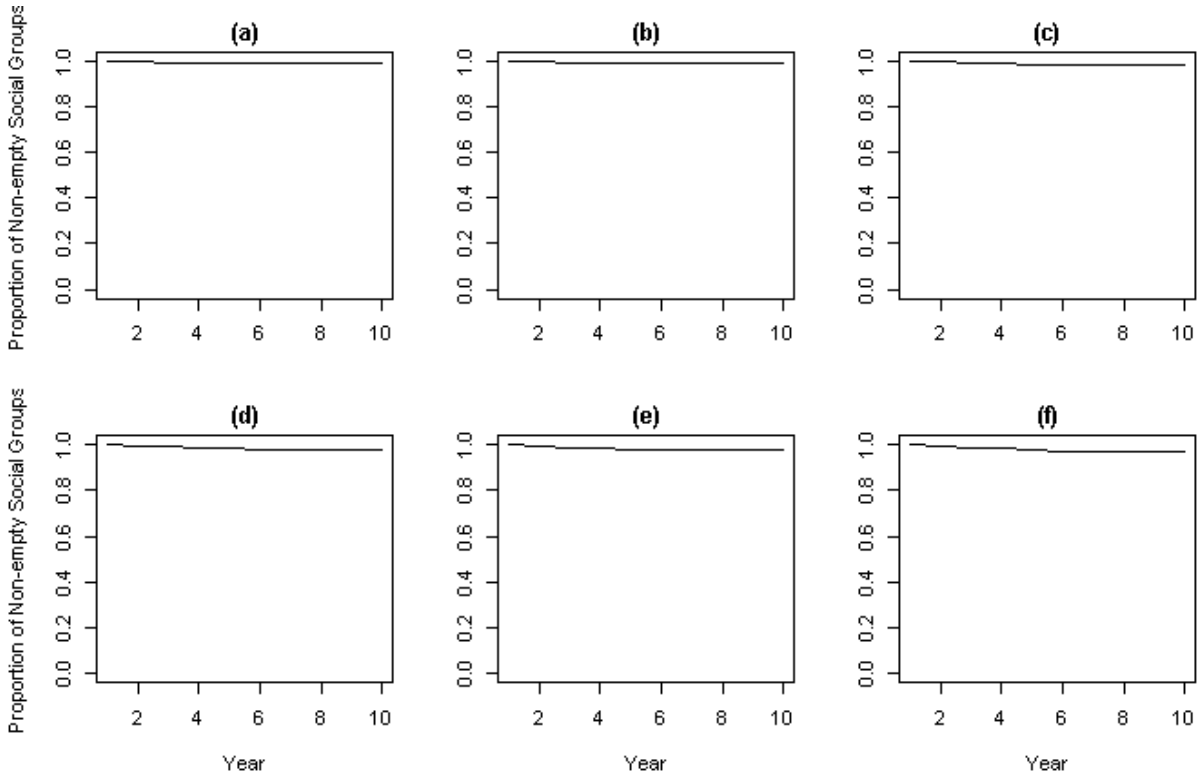


Figure 6.13: Mean annual proportion of non-empty social groups for frequency-dependent models with DIM at (a) 0, (b) 0.2, (c) 0.4, (d), 0.6, (e) 0.8, (f) 1.0.

Comparison simulations

Figures 6.14 and 6.15 show the population sizes after 200 years for all twelve models, with the first 100 years under the assumption of complete DIM, and the subsequent 100 years under the assumption of DIM values between 0 and 1. After 100 and 200 years, all models appear to have stabilised, either from their initial conditions (for the first 100 years) or from the system perturbation (for the second 100 years).

As in the main analyses, the group size definition used made little difference to the qualitative behaviour of the system for low and high DIM values, and the observed behaviours appeared to be most pronounced when just using adults for the group size definition (this can be observed in the main analyses from the graphs of Figures 6.4 and 6.5, with effects most pronounced in 6.4(c) and 6.5(c)).

Under both model transmission assumptions, DIM values of 0.8 and 1 resulted in at least half of the simulations giving a significant negative association between adult group size and *M. bovis* prevalence after 200 years (with the first 100 years under the assumption of complete DIM). In contrast there were no significant positive associations between adult group size and prevalence for DIM values of 0.8 and 1. Furthermore, for all smaller DIM values, there were not more significant negative associations between adult group size and prevalence than expected under the assumption of either transmission process.

Sensitivity analysis

All analyses using alternative parameter values (Tables 6.1 and 6.2) resulted in more significant negative association between adult group size and *M. bovis* prevalence than expected when complete DIM ($\alpha = 1$) was assumed, and none of the analyses under the same DIM assumption gave more significant positive associations between adult group size and *M. bovis* prevalence than expected. Furthermore when no DIM was assumed ($\alpha = 0$) there were never more significant negative associations between adult group size and prevalence than expected.

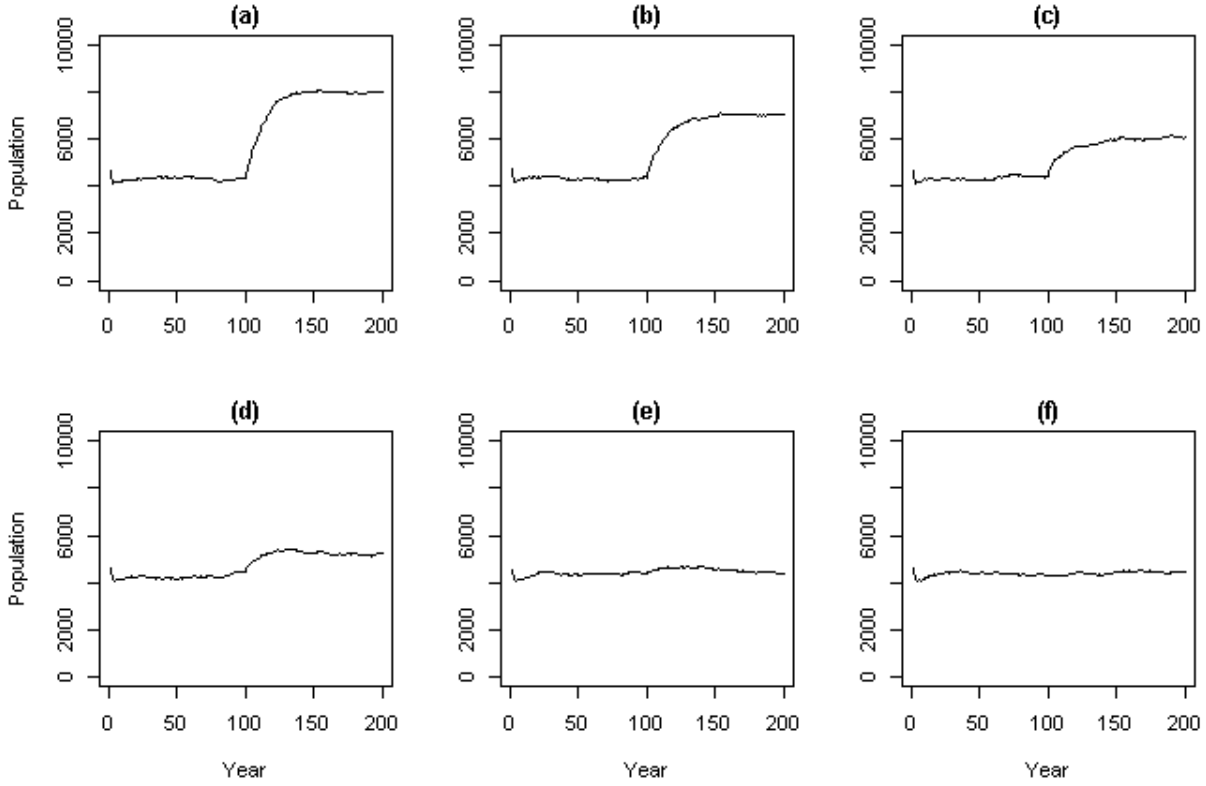


Figure 6.14: Mean annual population for density-dependent models with perturbation at 100 years from complete DIM to DIM equal to (a) 0, (b) 0.2, (c) 0.4, (d), 0.6, (e) 0.8, (f) 1.0.

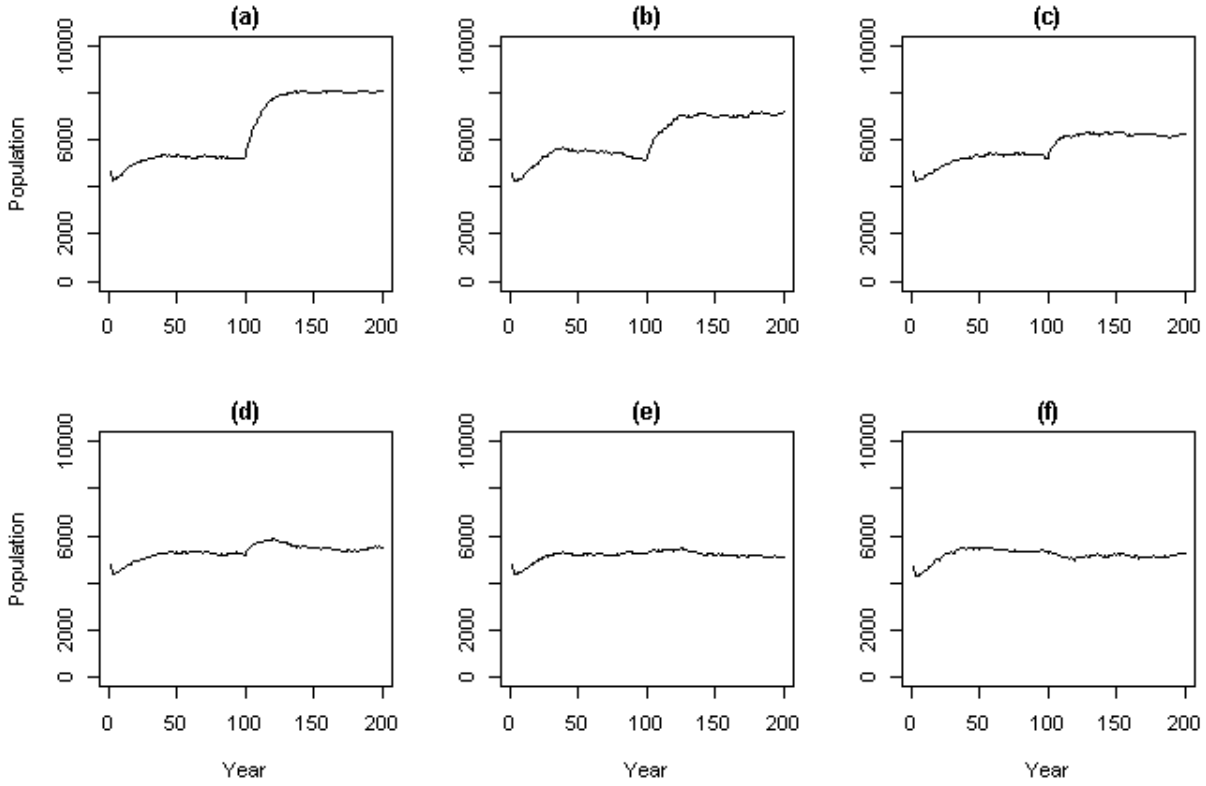


Figure 6.15: Mean annual population for frequency-dependent models with perturbation at 100 years from complete DIM to DIM equal to (a) 0, (b) 0.2, (c) 0.4, (d), 0.6, (e) 0.8, (f) 1.0.

Discussion

These results suggest that under the assumption of either density-dependent or frequency-dependent transmission, increasing the level of DIM would increase the probability of observing a significant negative association between social group size and prevalence, and reduce the probability of observing a significant positive association. This effect appears to be present at lower levels of DIM under the assumption of frequency-dependent transmission compared to models assuming density-dependent transmission, but this effect is strong at higher levels of DIM under both transmission assumptions. As expected, there were many simulations showing a significant positive association when DIM was 0 under either transmission assumption.

As DIM increases from 0 to 0.2 in the frequency-dependent models (Figure 6.5), there is a qualitative change from there being more positive associations than negative associations when DIM is 0 to the opposite when DIM is 0.2. This qualitative change only occurs in the density-dependent models (Figure 6.4) as DIM increases from 0.2 to 0.6. Therefore this qualitative change appears to occur at distinctly different DIM values depending on the transmission assumption.

The observation that the point of intersection for all six graphs in Figures 6.4 and 6.5 were above the 2.5% threshold is somewhat surprising, as under the assumption of random chance it would be expected that these lines would cross somewhere around this line. Even ignoring interpolated points of intersection, there were still DIM values for which there were a greater number of significant positive *and* negative associations between all groups size definitions and prevalence (when DIM = 0.4 for density-dependent transmission model results). One explanation for this could be that the system is sensitive to an element of initial stochasticity, which subsequently drives the system one way or the other, thus generating a greater number of significant associations in both directions.

Excluding younger individuals from the group size definition decreased the DIM value of the point of intersection for all six graphs of Figures 6.4 and 6.5. This could be due to the limit on the number of mothers that can successfully breed, which is set at three in these models. This means that in larger groups (in particular groups with more than three adult females), the proportion of cubs may be smaller than in smaller groups. Thus when cubs are included in the group size definition, the variation of the set of group size values decreases, consequently

dampening the size of the observed effect between group size and prevalence. When only yearlings and adults, and furthermore adults, are considered, the variation is larger, producing a larger effect, explaining why the DIM values at the point of intersection for Figures 6.4(c) and 6.5(c) are smaller than in Figures 6.4(a), (b) and 6.5(a), (b) respectively.

The qualitative behaviour of the proportion of significant negative associations between group size and prevalence in the comparative analyses, in which the simulations were run for 100 years under the assumption of complete DIM and then perturbed and run for a further 100 years, are in agreement with those found in the main analyses for both large and small DIM values. Furthermore, the sensitivity analyses further demonstrate the robustness of the original analyses, as all analyses using alternative parameter values resulted in an increase in the proportion of significant negative associations between group size and *M. bovis* prevalence, and thus mirrored the qualitative behaviour of the results obtained in the main analyses.

The level of DIM required in order to have a reasonable chance of replicating the trend of the significant negative associations found in Woodroffe *et al.* (2009) appears to be relatively low compared to the level estimated from natural badger populations (equivalent to DIM = 1 in this work, taken from Graham *et al.*, 2013). Summarising over the three graphs of Figure 6.4, the level of DIM at which it becomes more likely than not to observe a significant negative association between group size and prevalence (i.e. an odds value of one) is slightly over 0.5; similarly summarising over the three graphs of Figure 6.5, the corresponding level of DIM to achieve an odds value of one is slightly under 0.5. Therefore even if the estimated level of DIM found in Graham *et al.* (2013) is an overestimate, it is likely that the significant negative association observed between social group size and prevalence could still have been driven by DIM.

The increase in population sizes over time for lower DIM values (Figures 6.6 and 6.7) are to be expected, as the baseline mortality was fixed for all models. This would similarly explain the increases in non-zero group sizes and the proportion of non-empty social groups observed for lower DIM values (Figures 6.10-6.13). Although prevalence increases over time for lower DIM values under both transmission assumptions (Figures 6.8 and 6.9), these increases appear to be steeper when density-dependent transmission is assumed (Figure 6.8) compared to frequency-dependent transmission (Figure 6.9). This is likely to be due to the population

increase at lower DIM levels, and under density-dependent transmission will increase the risk of infection to susceptible individuals.

A potentially important application of these results is in understanding the relationship between group size and prevalence in badger populations with low levels of disease-induced mortality. This could be in a natural population, for example, one where resources are more available, and badgers are more resistant against the impact of *M. bovis* on their survival. Another example could be a badger population subjected to a vaccine that is able to suppress the progression and severity of infected badgers, such as Bacillus Calmette-Guérin (Chambers *et al.*, 2010). As shown in the results above, if disease-induced mortality is significantly reduced, a different relationship between group size and prevalence is more likely to be observed. However, transmission parameters may also be suppressed in such a population, and such a system would have to be modelled separately to ensure the same qualitative behaviours shown in this work are replicated. Furthermore, *M. bovis* infection may be harder to detect in these partially-vaccinated individuals.

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Chapter 7: Discussion

Summary of findings

The relationship between bovine TB, cattle and badgers (*Meles meles*) is complex, and is likely to require scientific insights from many different angles in order to make progress in understanding how to control the disease. The presence of *Mycobacterium bovis* within badger populations is known to play a crucial role in the problem of bovine TB in cattle. Therefore a thorough understanding of the biological and ecological dynamics of infected badger populations, together with clear evidence of how such a complex epidemiological system responds to control measures such as badger culling, is needed to ensure effective disease control. The work presented in Chapters 4 and 6 of this thesis explores theoretical aspects of both ecological and epidemiological dynamics of *M. bovis*-infected badger populations, whilst the results presented in Chapters 3 and 5 provide new insights into the effects of the proactive RBCT culls on the culled badger populations.

Chapter 3 uses a novel method to estimate the proportion of badgers culled during the RBCT proactive culls based on parentage assignments, and how these results compare to the culling efficacy criteria relating to government pilot badger culls in England. I estimated the efficacy of the initial proactive culls of the RBCT as 72.8% (95% CI: 65.6-79.8%), which was significantly higher than a previous estimate by Smith and Cheeseman (2007) of 59.9%. In their paper, Smith and Cheeseman (2007) extrapolate from a curve of cumulative daily culling totals in order to estimate the total number of badgers available to be caught prior to culling. Combining this estimate of the pre-RBCT culling population with data on the number of badgers culled during the initial culls, Smith and Cheeseman's estimates of the immediate badger population reduction for the initial proactive RBCT culls ranged between 32% and 77%. This wide range of values was based on the culling efficacy estimates for the initial proactive culls in each of the ten RCBT triplets, with seven of these estimates between 64-77% and the remaining three between 32-39%. Smith and Cheeseman (2007) cited individual reasons for the low estimates of these latter three triplets. My estimates of culling efficacy for the eight initial proactive culls of the RBCT analysed in Chapter 3 are much less varied (ranging between 56.3-85.1%) compared to the equivalent results of Smith and Cheeseman (2007) (ranging between 37.3- 76.6%). My estimates are based on methods using genetic

data, and therefore offer estimates that do not depend on any assumptions about the variability of culling effectiveness within the initial culling period.

Woodroffe *et al.* (2008) provide a separate analysis that estimates badger population reduction after several years of RBCT proactive culling, using indirect measures of badger abundance. The results of Woodroffe *et al.* (2008) showed a 73% reduction in badger latrine density, 69% reduction in the density of active badger burrows, and a 73% reduction in the density of badgers killed in road traffic accidents; these results were interpreted as an approximate badger abundance reduction of 70% for subsequent government culling policies (DEFRA, 2010). The distinction between the estimates of Woodroffe *et al.* (2008) and Smith and Cheeseman (2007) highlighted in this discussion should be made clear however, as the former relates to badger abundance reduction (and not culling efficacy) after several rounds of proactive RBCT culling, whereas the latter paper estimates the proportion of badgers culled during the initial proactive RBCT culls (and is thus a 'snapshot' estimate in comparison). The estimates of the badger culling efficacies of the follow-up proactive culls of the RBCT presented in this thesis are therefore the first of their kind, with mean culling efficacy estimated as 57.8% for adult females and 46.4% for adult males.

The inherent difficulty of accounting for the effect of perturbation in estimating parentage means that these estimates could be below the true culling efficacy values for adult females and adult males respectively. However the idea that perturbation affects this parentage-based methodology is consistent with the observation of a lower adult male culling efficacy estimate of 46.4% compared to the adult female culling efficacy estimate of 57.8%, as adult males are known to range more widely than adult females (Pope *et al.*, 2007). This is because both maternal and paternal assignments are based on parent-cub distance, and adult males are known to range more widely than adult females after culling (Pope *et al.*, 2007). Therefore it is likely that the true culling efficacies of the follow-up proactive culls of the RBCT are better estimated by the maternal estimate of 57.8% rather than the paternal estimate of 46.4%.

The estimates of culling efficacies outlined above relate to the proactive RBCT culls, which were performed on an approximately annual basis. Any disease management strategy based on repeated annual badger culling could be strongly affected by a new cohort of cubs each year, who could be infected or highly susceptible to *M. bovis*. Quantifying associations between parent and cub *M. bovis* infection status could therefore be of high importance in understanding how repeated culling may impact on the transmission of *M. bovis* infection in

badger populations. In Chapter 4 I therefore investigated the associations between the *M. bovis* infection status of cubs and parents, using the parentage assignments from Chapter 3. In particular, I assessed the evidence for pseudo-vertical transmission (transmission of *M. bovis* from mother to cub shortly after birth, whilst cubs are still dependent on their mothers) and for a genetic pre-disposition in susceptibility to *M. bovis* infection. The results presented here showed significant positive associations for both mother-cub ($p = 0.008$) and father-cub ($p = 0.008$) infection status, with odds ratios for maternal (6.553, 95% CI: 1.637-26.232) and paternal (4.581, 95% CI: 1.480-14.183) associations of the same degree of magnitude. As almost half of fathers are thought to not be in the same social group as the cub (Dugdale *et al.*, 2007), a significant association between cubs and fathers is unlikely to be due to transmission of *M. bovis* through close contact. Therefore these results give evidence for the existence of a genetic pre-disposition in susceptibility to *M. bovis* infection from both mothers and fathers to cubs. Any significantly increased risk of infection from having an infected mother compared to having an infected father might have suggested pseudo-vertical transmission was taking place (as well as a genetic predisposition), however the data used for these analyses did not reveal any significant difference. With improved genetic data, more high-confidence parentage assignments could be made, and would therefore decrease the width of the confidence intervals, allowing a more confident assessment on the differences between maternal and paternal *M. bovis* infection associations with cubs.

The existence of a genetic pre-disposition in susceptibility to *M. bovis* infection in badgers could potentially add more weight to the argument of selective badger culling, in which only badgers that are infected with *M. bovis* are culled on an annual basis. Any badger culling programme of this nature would decrease the proportion of infected individuals (potentially due to their genetic pre-disposition to *M. bovis* susceptibility), and would reduce the frequency of such a genetic susceptibility from being passed to future generations of offspring. A selective culling strategy may therefore artificially create future generations of badgers less susceptible to *M. bovis* if performed repeatedly. There are however major practical limitations at present to such a culling practice, including the lack of a live test for *M. bovis* infection in badgers with high sensitivity (Chambers *et al.*, 2008, Drewe *et al.*, 2010). Moreover, it is still unclear whether selective culling would avoid the detrimental effects of social perturbation (Bielby *et al.*, 2014), which may always be a barrier to the use of any sort of badger culling as a disease management strategy. Despite these practical limitations at present, the government time-scale for eradicating bovine TB in cattle is many

years away (DEFRA, 2014), and so understanding the impacts of culling practices that are not possible now but may be possible in the future is still of high importance.

An alternative strategy for managing *M. bovis* infection in badgers that would take advantage of the existence of a genetic predisposition in *M. bovis* susceptibility is selective sterilisation. As with the idea of selective culling outlined above, this would aim to remove infected badgers on an approximately annual basis, as individuals with a genetic susceptibility may be likely to be infected. This non-culling strategy would be likely to avoid the detrimental effects of social perturbation caused by culling, and could be more effective at reducing *M. bovis* levels in badger populations (Swinton *et al.*, 1997), through artificially producing future generations of badgers with lower susceptibility to *M. bovis*. However, such a strategy would only be able to reduce the proportion of individuals that are highly susceptible to *M. bovis* one generation at a time, and so the long-term nature of such a policy may be an unattractive option to policy makers.

Furthermore, the effects of annual selective sterilisation on the longer term population dynamics of badgers are unknown. For example, competition for breeding success amongst adult females within the same social group can be high, and adult females that are unsuccessful at producing a litter in a given year may move to another social group with less competition (Woodroffe and Macdonald, 1995). If sterilised adult females (assumed to be infected in order to be sterilised under such a strategy) were to leave their social group upon an unsuccessful breeding year due to being sterilised, this may potentially spread *M. bovis* infection to social groups that would have otherwise been uninfected. However, sterilisation may also have hormonal effects that suppress attempts to reproduce which might negate any potentially increased movement between social groups. As with selective culling, the viability of a strategy using selective sterilisation would also be dependent on the availability of an effective live test for *M. bovis* in badgers, as using a test with compromised sensitivity (Chambers *et al.*, 2008, Drewe *et al.*, 2010) could result in such a disease management strategy taking many generations of badgers, and many years of selective sterilisation operations, to significantly impact on *M. bovis* levels in badger populations.

As well as focussing on the epidemiological importance of the relationship between parents and cubs in Chapter 4, Chapter 5 uses the parentage assignments of Chapter 3 to investigate the humaneness of the proactive RBCT culls relating dependent cubs that had their mother culled. To best ensure that mothers of dependent cubs were not culled during the RBCT, no

culling was permitted during the months of February-April inclusive (a period termed the 'closed season'). Through using high-probability modal maternal assignments, the mean number of cubs culled per mother (based on having at least one cub assigned) for each month was calculated and compared with expected values based on survival and trappability rates from other populations. Previous work by Woodroffe *et al.* (2005a) estimated that a mean of nine unweaned cubs per year were missed during the RBCT culls, however these estimates were not attained using parentage assignments from genetic data. Therefore the analyses presented in this thesis were aimed at using all currently available information, the results of which showed no evidence of cubs being missed in May. Therefore there was no evidence that the closed season, as defined for the RBCT, needs to be extended to include May in relation to culling mothers of dependent cub for any future badger culling operations. One application of this result relates to the closed season periods defined for the pilot badger culls that began in 2013. The closed season of the pilot culls for both cage-trapping and free shooting includes May, in contrast to the closed season of the RBCT.

Efforts to minimise potential suffering to badger cubs through avoiding the culling of mothers with dependent cubs have been in place ever since the government changed the method of badger culling from gassing to cage-trapping in 1982 (Dunnet *et al.*, 1986). Between 1982 and 1997, any adult females that upon capture were judged to be lactating were assumed to have dependent cubs and so were released (Krebs *et al.*, 1997) in order to avoid their cubs suffering in such a manner. However detecting whether a female is lactating is not always an accurate process. During the 1996-1998 period prior to the RBCT, 4.3% of 1363 adult females culled using cage-traps were found to be lactating (Woodroffe *et al.*, 2005a), meaning that these females may have had dependent cubs. Furthermore, cubs may still be dependent on their mothers for survival after they are no longer dependent on their mother's milk, for example whilst learning how to forage from their mother. Therefore the cubs of a mother that is not lactating are not necessarily independent of their mothers for survival. These limiting factors to lactation-based adult female culling was therefore the motivation for developing the closed season (Bourne *et al.*, 2007).

The issue of humaneness has long been an important aspect to badger culling strategies (Bourne *et al.*, 2007, Dunnet *et al.*, 1986), and although most badger culling practices carried out in England are designed to minimise suffering, it is often impossible to avoid any sort of suffering. However, there are many ways in which culling can cause suffering to badgers, and there is often a huge variation in the level of potential suffering between different culling

methods too. The potential suffering that can be experienced by cubs starving or dying from dehydration due to their mother being killed is likely to be relatively high, and so is an important issue relating to badger welfare. The method of cage-trapping, as used to cull badgers during the RBCT, also has other sources of potential suffering for badgers, including teeth, jaw and skin damage whilst trapped in the cages (although this is was judged to happen to a small minority of individuals during the RBCT, Woodroffe *et al.*, 2005b). Although measures of physical damage to badgers from the RBCT were assessed (Woodroffe *et al.*, 2005a, Woodroffe *et al.*, 2005b), no measures of stress were assessed through monitoring the behaviour of trapped individuals (Woodroffe *et al.*, 2005b). It is possible that the stress of being trapped in cages could be used as a humaneness argument against cage-trapping.

In more recent years new concerns have been raised about the humaneness of badger culling. Analyses of the pilot badger culls of 2013 measured the humaneness of free shooting based on whether more than 5% of badgers shot were estimated to have taken more than 5 minutes to die. The results of these analyses showed that this target was not met, with the number of badgers taking longer than 5 minutes to die estimated to be between 7.4-22.8% (Munro *et al.*, 2014). In 2014 the government decided to continue using the method of free shooting without any independent monitoring (of humaneness or of culling efficacy); the Badger Trust subsequently appealed against this decision, however this appeal was unsuccessful.

To complement the application-driven findings of Chapters 3, 4 and 5, Chapter 6 presents a more theoretical insight into how *M. bov*is infection might play an important role in badger population dynamics. Analyses of data from the RBCT showed a consistently significant negative association between badger social group size and *M. bovis* prevalence in the trial areas, and one hypothesised explanation for this was that disease-induced mortality might suppress group size (Woodroffe *et al.*, 2009). Therefore I developed an individual-based stochastic simulation model to explore the relationship between badger social group size and *M. bovis* prevalence with varying degrees of disease-induced mortality.

Model simulations showed that a significant negative association between badger social group size and *M. bovis* prevalence could be observed when disease-induced mortality was between 20% and 100% of that observed in studied badger populations (Graham *et al.*, 2013). For models in which *M. bovis* transmission was assumed to be frequency-dependent (in which the probability of becoming infected is dependent on the proportion of infected individuals in the same social group), a disease-induced mortality of just 20% of that found in

natural badger populations was required to replicate this negative association, whereas when transmission was assumed to be density-dependent (in which the probability of becoming infected is dependent on the number of infected individuals in the same social group), a disease-induced mortality of at least 40-60% of that found in natural badger populations was required to replicate such an negative association.

The original finding of a negative association between badger social group size and *M. bovis* prevalence by Woodroffe *et al.* (2009) was surprising, as previous work on general epidemiological dynamics had predicted that large groups would allow more infectious contacts, and thus result in a higher prevalence (Anderson and May, 1979). This theory was backed by strong positive associations between host species group size and prevalence of contagious parasites in other wildlife species (Cote and Poulin, 1995). However it is likely that farmed cattle play an important role in the dynamics of *M. bovis* infection in badgers, potentially voiding any simplistic general epidemiological dynamics theory being applied to modelling such a wildlife disease.

Wider implications and future work

There is no clear consensus on the best method for controlling *M. bovis* infection in badgers in relation to managing bovine TB in cattle. Even within the UK and the Republic of Ireland there are currently four different active policies relating to badgers and bovine TB, with many more in the past too. In England there were pilot culls based on free shooting and cage-trapping in West Gloucestershire and West Somerset in 2013, as well as the Badger Vaccine Deployment Project (a five year project starting in July 2010 in an area of Gloucestershire). Wales is in their third year of a five year badger vaccination scheme in an area of high bovine TB levels, known as the Intensive Action Area. In Northern Ireland a Test and Vaccinate or Remove (TVR) research project began in June 2014, in which badgers are trapped and tested for *M. bovis*; if they test positive they are killed, and if they test negative then they are vaccinated. In the Republic of Ireland badger culling based on the use of snares (banned for use on badgers in the UK) has been employed for many years. Understanding the effects of badger culling and vaccination programmes, together with the population and infection dynamics of *M. bovis*-infected badger populations, is therefore of key importance.

The culling efficacy estimate for the initial culls of 72.8% increases the confidence in the 70% culling efficacy criterion set out by the English government for the 2013 pilot culls, whilst the estimate for the culling efficacies of the follow-up culls of 57.8% (based on

maternal assignments) is the first of its kind, and could be used in combination with the initial cull estimate to inform policy. Given that the culling efficacy of the initial culls (i.e. the immediate population reduction) was estimated to be approximately 70%, and the population reduction after several RBCT culls was also estimated to be approximately 70% (Woodroffe *et al.*, 2008), it could be that the estimate of the culling efficacy for the follow-up culls of 57.8% also represents an estimate for the approximate annual culling efficacy required to maintain a population reduction of 70%. Although these estimates could be used to inform any possible future wide-scale badger culls based on cage-trapping (as in the RBCT), it is unlikely to inform the pilot culls of 2014 and onwards, as the culling efficacy of the method of free shooting badgers during the English pilot culls of 2013 was shown to be much lower than 70% (Munro *et al.*, 2014).

Alternative badger culling methods such as gassing, poisoning and snaring may possibly have high culling efficacy levels of near 70%, however these methods are all currently illegal in the UK, and would also potentially have a high cost to badger welfare (Bourne *et al.*, 1998, Dunnet *et al.*, 1986). Therefore given the substantially lower culling efficacy estimates for free shooting compared to cage-trapping, it is likely that cage-trapping is the most effective legal method of badger culling at attaining maximal culling efficacy.

The results of Chapter 4 suggest that selective sterilisation, or selective badger culling (as currently being trialled in Northern Ireland), might target individuals that are genetically more susceptible to *M. bovis*, and who may be more likely to produce cubs which are also more susceptible to *M. bovis* in the absence of any disease management strategy. Such strategies might therefore have the potential to target generational persistence of *M. bovis* infection in badger populations through reducing the frequency of highly susceptible individuals in future generations. However as outlined above, current practical limitations relating to live test sensitivity for *M. bovis* in badgers (Chambers *et al.*, 2008, Drewe *et al.*, 2010) represent major barriers in being able to confidently implement such strategies at present (Woodroffe *et al.*, 1999). Furthermore, for selective culling there is the possibility that removing just a small proportion of badgers could still prompt social perturbation (Bielby *et al.*, 2014) and thus potentially increase the spread of *M. bovis* in badger populations. Therefore future research towards improving the sensitivity of a live test for badgers, as well as behavioural studies into the effects of selective badger removal on badger ranging behaviour (as suggested in Bielby *et al.*, 2014), would be needed in order to know if

either selective sterilisation or selective culling of badgers could help reduce bovine TB levels in cattle.

Despite previous hypotheses (Anderson and Trewhella, 1985, Smith *et al.*, 1997), there was no evidence of pseudo-vertical transmission found from the results of Chapter 4. Any evidence for the existence of pseudo-vertical transmission would have prompted concern over the potential effectiveness of injectable *M. bovis* vaccines (Chambers *et al.*, 2010) for badger cubs. If a high number of cubs become infected through pseudo-vertical transmission of *M. bovis* from their mother before they first emerge from their setts, then the impact of any vaccination programme using injectable vaccines (which can only vaccinate cubs once they emerge) might be severely limited. If this were the case, then recommendations for future research might have been directed towards the idea of an orally administered vaccine that could be placed down setts, and potentially consumed by cubs prior to emerging from their setts for the first time. However given the lack of evidence for pseudo-vertical transmission presented in this work, there is no reason to suggest that injectable vaccination programmes, as currently taking place in England (DEFRA, 2014) and Wales (WAG, 2014), would be undermined by such an infection transmission process.

As well as examining the epidemiological significance of pre-emergent badger cubs through pseudo-vertical transmission in Chapter 4, the significance of pre-emergent badger cub welfare is the main theme of Chapter 5. Whilst the results of Chapter 5 found no evidence to suggest that the closed season of the RBCT needs to be extended to include May in order to ensure that the killing of mothers does not impact badger cub welfare, the lack of evidence of pseudo-vertical transmission in Chapter 4 would also suggest that, even in the absence of badger welfare considerations, there is no disproportionate epidemiological benefit of culling mothers of dependent cubs during the closed season months compared to later months of the year. Therefore there is also no evidence to suggest that the closed season needs to be shortened on epidemiological grounds. Future research into infection associations between parents and cubs, or more generally between any individuals, using whole genome sequencing data on *M. bovis* infections would provide far greater resolution in investigating any transmission processes such as pseudo-vertical transmission. The power of this method of analysis has already been demonstrated in linking infections between badgers and cattle in Northern Ireland (Biek *et al.*, 2012), and is likely to provide more evidence on *M. bovis* transmission processes in badgers.

Understanding the processes that underpin the observed population and disease dynamics in *M. bovis*-infected badger populations is of key importance in managing *M. bovis* infection in badger populations, and bovine TB in cattle. For example, understanding the changes in badger social structure in response to culling (social perturbation) helped to explain the detrimental effects of both proactive and reactive badger culling on bovine TB levels during the RBCT, and thus allows more informed policy decisions (Pope *et al.*, 2007, Woodroffe *et al.*, 2006). The work presented in Chapter 6 of this thesis presents a plausible hypothesis to explain the observed association between badger social group size and *M. bovis* prevalence (Woodroffe *et al.*, 2009) through the fact that even relatively low levels of disease-induced mortality could potentially suppress group size in infected groups. Many previous hypotheses of a positive association between group size and prevalence had focused on a reverse causal argument that larger social groups allow more infectious contacts, and thus increase prevalence (Anderson and May, 1979, Cote and Poulin, 1995). This may be true of infections in other social host species, particularly in which disease-induced mortality is low and thus does not influence group size as much. Similar modelling work could therefore be applied to other populations of infected animals to analyse relationships between population abundance, infection prevalence and disease-induced mortality.

Overall, the findings and insights presented in this thesis span many important theoretical and applied aspects of the epidemiology of *M. bovis* infection in badger populations. Managing bovine TB is a highly complex problem, with many important issues not relating to badgers. However, badgers clearly do play a crucial role in the problem of bovine TB, and this work makes significant progress in further understanding this role. This discussion chapter also gives suggested directions for future research to further the insights presented in this thesis, and more generally to further improve our understanding of what disease management strategies relating to badgers may be of most benefit.

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