Heterogeneity in the risk of *Mycobacterium bovis* infection in European badger (*Meles meles*) cubs

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SUMMARY

The behaviour of certain infected individuals within socially structured populations can have a disproportionately large effect on the spatio-temporal distribution of infection. Endemic infection with *Mycobacterium bovis* in European badgers (*Meles meles*) in Great Britain and Ireland is an important source of bovine tuberculosis in cattle. Here we quantify the risk of infection in badger cubs in a high-density wild badger population, in relation to the infection status of resident adults. Over a 24-year period, we observed variation in the risk of cub infection, with those born into groups with resident infectious breeding females being over four times as likely to be detected excreting *M. bovis* than cubs from groups where there was no evidence of infection in adults. We discuss how our findings relate to the persistence of infection at both social group and population level, and the potential implications for disease control strategies.

Key words: Tuberculosis (TB), veterinary epidemiology.

INTRODUCTION

Bovine tuberculosis (TB), caused by *Mycobacterium bovis* is a zoonotic disease with a significant impact on livestock health and production in the UK [1]. Endemic infection in Eurasian badger (*Meles meles*) populations has been linked to the persistence of infection in cattle in parts of Great Britain [2] and Ireland [3].

In recent years there has been an increasing recognition of the importance of social structure and individual behaviour in understanding the epidemiology of infectious disease in wildlife populations [4, 5].

For example, studies of white-tailed deer (*Odocoileus virginianus*) in Michigan, USA, have demonstrated higher risks of *M. bovis* transmission among closely related individuals, with infected deer being more closely related to each other than uninfected deer [6]. In addition, belonging to an infected social group has been shown to be a risk factor for *M. bovis* infection in wild boar (*Sus scrofa*) and red deer (*Cervus elaphus*) in the Doñana National Park in Spain [7]. Furthermore, certain individuals may play a disproportionately important role in both the transmission and maintenance of infection in a population [8]. The epidemiology of *M. bovis* in badger populations has been well studied, in particular in a free-living high-density population at Woodchester Park in south-west England, which has been intensively monitored since 1981. In moderate to high-density badger

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populations individuals are organized into social groups occupying relatively discrete, contiguous territories [9]. Factors associated with the persistence of infection in the Woodchester Park population include this well-defined social structure which leads to disease clustering [10, 11], pseudo-vertical transmission from dam to offspring [10, 12], and the longevity of potentially infectious individuals [12].

The annual breeding cycle of the badger ensures the relatively synchronized production of a cohort of susceptible juveniles into the population each year, with the peak of births in early-mid February in the south of England [9], and an average litter size of 2·7 [13]. The infection status of other group members, and in particular that of the breeding females, are likely to be important determinants of the risk of infection in cubs. Until about age 8 weeks, badger cubs remain largely underground in the sett [9]. When they first emerge (usually in mid April in southern England) cubs only spend short periods of time above ground, staying close to the entrance holes and having little contact with adults other than their (assumed) dam [14]. In the absence of genetic data, parentage in observational studies is necessarily assumed, although alloparental care by other unsuccessful adult females may occur [15, 16]. Up to age ~16 weeks, cubs spend an increasing amount of time above ground, emerging earlier and interacting more with other social group members. It appears likely therefore that a cub's social network is restricted in early life, but thereafter expands and develops during the summer months.

It has already been demonstrated that infectious female badgers are able to reproduce successfully and transfer infection to their offspring [12], and that infectious female badgers continue to breed for several years with no detectable significant negative effect on reproductive success [17]. In addition, the presence of infectious adult females in a social group has been shown to be a significant risk factor for the detection of *M. bovis* excretion in cubs [10]. We propose that the likelihood of a cub acquiring infection early in life should follow a gradient of risk, being highest in social groups containing infected reproductively active females, intermediate in those with other infected adults, and lowest in groups with no evidence of infected adults.

Information on the major transmission pathways and their relative contribution to observed epidemiological patterns is likely to be valuable in the formulation of effective disease control policies. The purpose of our analyses was to assess the risk of cub infection relative to the infection status of resident adults in their natal social group, using data from a long-term study of naturally infected badgers in a high-density population in south-west England.

METHODS

Badger life history data

Badger life history data from 1982 to 2011 inclusive, including *M. bovis* infection status, were obtained from the live trapping and sampling study at Woodchester Park in Gloucestershire, south-west England. The study site consists largely of pasture and mixed woodland, supporting 36 badger social groups [10]. The boundaries of social group territories were estimated on an annual basis using a bait-marking technique [18].

Traps were deployed at each sett four times a year (twice from 1982 to 1984) (for details see [11]), but no trapping took place from February to April inclusive to minimize the likelihood of capturing females in early stages of lactation, or dependent cubs. All badgers were transported to a dedicated sampling facility where they were examined and sampled under general anaesthesia [11]. Females were considered to be reproductively active if visual examination and palpation of the mammary glands and the surrounding area were consistent with previous or current lactation. Badgers were considered to be cubs for a full year, based on a nominal median birth date of mid-February [9]. A blood sample was taken and the serum was drawn off for serological assays to detect the presence of antibodies to *M. bovis* using the Brock ELISA test [19], and from July 2006 using the more recently developed Brock TB Stat-Pak (SP) test [20]. Moreover, from July 2006, a second blood sample collected into lithium heparin anticoagulant was used to measure the cell-mediated response to *M. bovis* using the quantitative interferon-gamma (IFN-γ) assay [21]. Clinical samples (sputum, faeces, urine, abscess, wound swabs) were collected for mycobacterial culture [22] to identify animals excreting *M. bovis*.

Cub infection status

Our analyses aimed to investigate associations between the infection status of cubs and adults (as derived from diagnostic test results) in each social group. Each cub was classified as positive or negative separately for each of the diagnostic tests. A cub was

Table 1. *Hierarchical categorization of each social group in each year for dataset A (badger captures from 1982 to 2005), based on the infection status of all adults and reproductive status of adult females captured in the group from May to the following January*

Social group infection status	Adult captures from May to the following January
Negative	All negative
Seropositive	At least one of seropositive status (excluding reproductively active females)
Excretor	At least one of excretor status (excluding reproductively active females)
Breeding seropositive	At least one reproductively active female of seropositive status
Breeding excretor	At least one reproductively active female of excretor status

considered positive for a specific test if one or more of its capture events yielded a positive test result. To increase the temporal and immunological scope of our study, we used two datasets of cub captures. The first (dataset A) consisted of 4275 captures of 1815 cubs from 1982 to 2005 inclusive (range for number of cubs per annum = 20–134, mean = 76). At each capture event at least one clinical sample was taken for *M. bovis* culture, resulting in an average of 2·4 clinical sampling events per cub. Of the 1815 cubs, 1761 had at least one blood sample taken for serological testing, resulting in an average of 2·3 serological tests per cub. Analysis of this dataset investigated associations between the infection status of adults in the natal group and the likelihood of a cub being detected as either seropositive (using the Brock ELISA test), or as excreting *M. bovis* (as indicated by a positive culture result). Results after 2005 were excluded from this dataset due to an apparent change in the performance of the Brock ELISA test [23]. The second dataset (dataset B) consisted of 609 captures of 266 cubs from May 2006 to January 2011 inclusive (range for number of cubs per annum = 25–66, mean = 53). All 266 cubs had at least one SP test and one IFN-γ test. On average each cub had 2·3 SP tests and 2·2 IFN-γ tests. Analysis of this dataset investigated associations between the infection status of adults in the natal group and the likelihood of a cub being detected as seropositive (using the SP test), or as IFN-γ positive. The IFN-γ assay has a superior sensitivity to the serological assays [24], and as a measure of cell-mediated responses, is likely to

be triggered earlier in the course of infection than a serological response [25].

Natal group infection status and cub infection risk

Each social group in each year was allocated to a category based on the infection status of the adults captured there from May to the following January. This effectively represented the natal environment for each cohort of cubs in a group. A natal group was assigned to each cub based on the social group in which it was first captured. First, infection status for each adult capture event was classified according to a one-way progressive system, using results of serological tests and the culture of clinical samples. Similar to the system described by Delahay *et al.* [10], and based on models of the immunopathogenesis of *M. bovis* infection in badgers [25, 26], individuals moved from 'negative' (all tests negative) to 'seropositive' (seropositive, culture negative), to 'excretor' (seropositive or seronegative, and culture positive) status. This method of classification is constrained by properties of the diagnostic tests, in particular the low sensitivity of both serological tests (about 54% for both tests [24]) and the culture of clinical samples from live badgers (25% [27] and 27·5% [28]). However, increases in sensitivity were gained by multiple testing, with an average of two captures per badger in any one year in this population [10], combined with an increase in serological test sensitivity as infection progresses [24]. In contrast, test specificity values of over 90% for the serological tests [20] and close to 100% for mycobacterial culture [27], ensured that social group infection status misclassification was consistent in the direction of underestimation. Over the period 1982–2005 (dataset A), each of 521 social group-year combinations was assigned to one of five categories of infection status (Table 1), based on over 6000 adult capture events (range for number of social groups per annum = 10–30, mean = 22). Over the period 2006–2011 (dataset B), each of 82 social group-year combinations was assigned to one of three categories of infection status (Table 2), based on over 1500 adult badger capture events (range for number of social groups per annum = 13–18, mean = 16). Group infection status for both datasets was based on the infection status of adults captured during the respective time periods. For some individuals captured prior to 1982 (dataset A), or 2006 (dataset B), infection status incorporated test results prior to the study periods for detecting infection in the cubs. We decided

Table 2. *Hierarchical categorization of each social group in each year for dataset B* (*badger captures from 2006 to 2011*), *based on the infection status of all adults and reproductive status of adult females captured in the group from May to the following January*

Social group infection status	Adult captures from May to the following January
Negative	None of excretor status
Excretor	At least one of excretor status (excluding reproductively active females)
Breeding excretor	At least one reproductively active female of excretor status

to exclude adult, and therefore social group serological status classification, from dataset B due to the absence of any serological results using the SP test prior to July 2006 and the change in performance of the Brock ELISA test after 2005, rendering the results after this time incomparable with those prior to 2005.

Logistic regression models were used to investigate associations between whether a cub was detected as infected using the Brock ELISA test or *M. bovis* culture (dataset A), and the IFN-γ assay or the SP test (dataset B), and the infection status of its natal social group. Each model used the test outcome (positive or negative based on all cub captures) for a cub as the response variable. Explanatory variables were the natal social group infection category (five levels for dataset A, three levels for dataset B), cub sex, and the number of times each cub was tested (log transformed).

Since no cubs were detected as excreting from 2006 to 2011, we were prevented from investigating factors associated with the likelihood of *M. bovis* excretion in cubs for dataset B. On the basis of previous observations of differing IFN-γ test performance in cubs and adults [24], the IFN-γ results from cub captures were reclassified, using the optical density (OD) value of the difference between the average responses to bovine and avian tuberculin. This involved using a cut-off value of 0·023 instead of the standard 0·044.

All statistical analyses were performed using Genstat 14th edition (VSN International, UK). Statistical significance was attributed to test results when $P < 0.05$.

RESULTS

The proportion of cubs detected as seropositive each year (using the Brock ELISA from 1982–2005 and the SP from 2006) remained between 5% and 10%

during the 1980s and early 1990s, increased steadily to 28% in 2000, and varied from 2000 onwards (Fig. 1). The proportion of cubs detected as infected using the more sensitive IFN-γ assay from 2006 onwards, also fluctuated from a minimum of 12% in 2006 to 37% in 2009. During the whole study period from 1982 to 2010 inclusive, the annual proportion of social groups with a resident breeding excretor female fluctuated, but did not exceed 30% in any given year (Fig. 1). Early in the study period, and in 2006, there were no captures of reproductively active females with evidence of *M. bovis* excretion. For dataset A from 1982 to 2005, the probability of a cub being detected as Brock ELISA test positive or *M. bovis* culture positive was significantly associated with the infection status of its natal social group. Cubs from excretor, breeding seropositive and breeding excretor groups were three, five and eight times, respectively, more likely to be seropositive than those from negative groups (P always < 0.05, Table 3). There was, however, no difference in the likelihood of detection of a seropositive response between cubs from seropositive groups and those from negative groups ($P > 0.05$). Cubs from breeding excretor groups were four times as likely to be detected as excreting compared to cubs from negative groups ($P < 0.05$, Table 4). For both the Brock ELISA test and culture, the probability of a positive result increased with the number of test events (P always < 0.05, Tables 3 and 4).

For dataset B, from 2006 to 2011, the probability of a cub being detected as IFN-γ or SP positive was also significantly associated with the infection status of its natal social group. Cubs from excretor and breeding excretor groups were respectively two and six times more likely to be IFN-γ positive than those from negative groups (Table 5), and cubs from breeding excretor groups were nearly eight times more likely to be SP positive than those from negative groups (Table 6). However, there was no significant difference in the likelihood of a SP-positive result between cubs from excretor groups and those from negative groups ($P > 0.05$, Table 6). For both response variables, the probability of a positive result did not significantly increase with the number of test events ($P > 0.05$, Tables 5 and 6).

DISCUSSION

Individual behaviour and social organization in wild animal populations can have a significant influence on the spatio-temporal distribution of infection [5],

Table 3. *Results from a logistic regression investigating the influence of natal group infection status on the likelihood of badger cubs being detected as seropositive using the Brock ELISA (n = 1761 tested from 1982 to 2005 inclusive)*

Parameter	Reference level	Estimate	OR (95% CI)	*P* value
Seropositive group	Negative	0·275	1·316 (0·653–2·654)	0·443
Excretor group	Negative	1·189	3·282 (1·815–5·937)	**<0·001**
Breeding seropositive group	Negative	1·665	5·287 (3·190–8·762)	**<0·001**
Breeding excretor group	Negative	2·132	8·436 (5·038–14·120)	**<0·001**
Number of serological tests (log transformed)	n.a.	1·209	3·350 (2·382–4·712)	**<0·001**
Sex	Female	0·008	1·008 (0·728–1·395)	0·963

OR, Odds ratio; CI, confidence interval; n.a., not applicable.
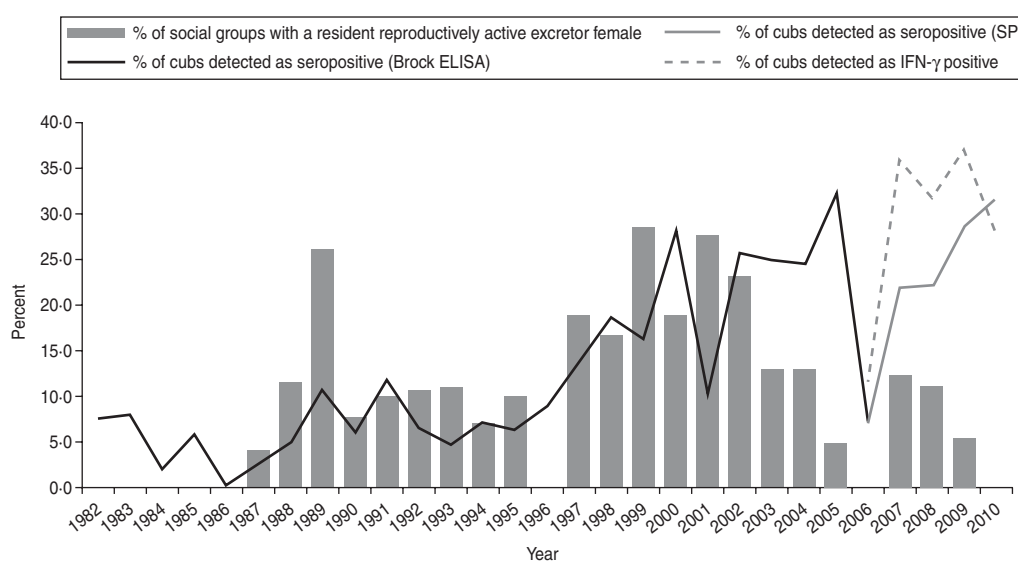Model deviance = 169, D.F. = 6, *P* < 0·001.

Fig. 1. Annual fluctuations in the percentage of social groups with a resident breeding excretor female badger from 1982 to 2010 inclusive, with the percentage of cubs detected as seropositive [Brock ELISA 1982–2005, Stat-Pak (SP) from 2006] and IFN-γ positive (from 2006 only) superimposed.

and certain individuals or groups may have a disproportionate influence on epidemiological processes [8]. In the present study we have used empirical data to assess the relative contribution of adult badgers of differing reproductive and infection status, to the acquisition and progression of infection in cubs born into the same social group.

We hypothesized that the risk of cub infection would be higher where there was evidence of the presence of *M. bovis* excretion in resident adults in the natal group, and that this risk would be greatest in the presence of infectious breeding females. Our analyses from both datasets supported these predictions. Cubs that were born into groups with evidence of *M. bovis* excretion in adults were more likely to be detected as IFN-γ positive, seropositive and as excreting

M. bovis than those born into groups with no such evidence in resident adults. In addition, cubs that were born into groups with reproductively active excretor females were the most likely to be detected as infected (regardless of diagnostic test). This is consistent with the close and prolonged contact a cub would have with its dam, and the limited social contact it would have with other group members in the first few months of life [14]. It is also possible that cubs may be particularly susceptible to infection during early life when their immune systems are likely to still be maturing (as observed in domestic dogs and cats [29]).

The prevalence of cub infection (as detected by serological tests and the IFN-γ assay) varied annually, reaching a maximum of 37% (detected as IFN-γ positive) in 2009 (Fig. 1). In addition, we found that the

Table 4. *Results from a logistic regression investigating the influence of natal group infection status on the likelihood of badger cubs being detected as excreting* M. bovis *by clinical sampling (n = 1815 cubs tested from 1982 to 2005 inclusive)*

Parameter	Reference level	Estimate	OR (95% CI)	*P* value
Seropositive group	Negative	0·421	1·524 (0·427–5·446)	0·517
Excretor group	Negative	0·980	2·665 (0·846–8·395)	0·094
Breeding seropositive group	Negative	1·001	2·722 (0·977–7·581)	0·055
Breeding excretor group	Negative	1·497	4·468 (1·625–12·290)	**0·004**
Number of cub captures with a culture result (log transformed)	n.a.	1·302	3·676 (1·784–7·578)	**<0·001**
Sex	Female	−0·115	0·892 (0·462–1·720)	0·732

OR, Odds ratio; CI, confidence interval; n.a., not applicable.
Model deviance = 28·6, D.F. = 6, *P* < 0·001.

Table 5. *Results from a logistic regression investigating the influence of natal group infection status on the likelihood of a badger cub being detected as IFN-γ positive (n = 266 cubs tested from July 2006 to January 2011 inclusive)*

Parameter	Reference level	Estimate	OR (95% CI)	*P* value
Excretor group	Negative	0·712	2·038 (1·101–3·773)	**0·023**
Breeding excretor group	Negative	1·865	6·453 (2·404–17·32)	**<0·001**
Total number of cub tests (log transformed)	n.a.	0·362	1·436 (0·784–2·631)	0·242
Sex	Female	0·308	1·361 (0·783–2·367)	0·275

OR, Odds ratio; CI, confidence interval; n.a., not applicable.
Model deviance = 19·6, D.F. = 4, *P* < 0·001.

Table 6. *Results from a logistic regression investigating the influence of natal group infection status on the likelihood of a cub being detected as Stat-Pak positive (n = 266 cubs tested from July 2006 to January 2011 inclusive)*

Parameter	Reference level	Estimate	OR (95% CI)	*P* value
Excretor group	Negative	0·553	1·738 (0·817–3·700)	0·151
Breeding excretor group	Negative	2·046	7·738 (2·853–20·980)	**<0·001**
Total number of cub tests (log transformed)	n.a.	−0·413	0·661 (0·319–1·370)	0·266
Sex	Female	0·222	1·248 (0·629–2·437)	0·516

OR, Odds ratio; CI, confidence interval; n.a., not applicable.
Model deviance = 16·6, D.F. = 4, *P* = 0·002.

percentage of the highest risk social groups in the population (i.e. those containing reproductively active female excretors) also varied on an annual basis, but never exceeded 30% (Fig. 1). In the face of limitations in serological test sensitivity (54% [24]), we acknowledge that cub seroprevalence estimates will be lower than the true prevalence, although on average all cubs were tested more than twice, thereby increasing the sensitivity of detection.

Our results build on previous research on the Woodchester Park badger population which reported an increased risk of disease detection in cubs in groups where an excreting adult female was also present [10]. They are also consistent with evidence from another population where unvaccinated cubs in vaccinated social groups were over four times more likely to be detected as infected at first capture when their natal group included at least one excretor badger [30]. However, the present study goes further by identifying evidence of an infection risk gradient for cubs where reproductively active excretor females posed the greatest risk, followed by seropositive breeding females,

other excreting adult badgers, other seropositive adult badgers and finally adults with no evidence of infection. This provides further evidence that the transmission of infection to young cubs by infected dams is likely to be important in the observed persistence and clustering of infection within badger social groups [10, 12]. In addition, it suggests that despite inherent limitations in the performance of the diagnostic tests employed in this study, they do provide biologically meaningful information on the relative risks of infection posed by different individuals.

We acknowledge that our classification of social group infection status underestimates the true status of groups, particularly if they included adults with recently acquired infection. This was likely to be the case despite the increase in sensitivity associated with multiple test events, and the increase in sensitivity of the serological tests with the progression of infection [24]. In addition, interpretation of the results from the serological tests of cubs in our study (i.e. the Brock ELISA and SP tests) should take account of the possibility of colostrally derived antibody being present in cubs, which is indistinguishable from that produced by a cub in response to natural infection. As reported for many other mammals, maternal antibody transfer is highly likely to occur in badgers, although there are no published data. In another mustelid, the ferret (Mustela putorius furo), the half-life of maternally derived antibodies is approximately 10 days with little detectable serum antibody by age 10–12 weeks [31]. It is therefore possible that colostrally derived antibody may be detectable during the summer months in late-born cubs. However, most cubs would be >10 weeks old by the start of the trapping season in May (based on a peak of births in mid-February [9]), hence if colostral antibody responses occurred they are likely to represent only a small proportion of the seropositive cub responses in our dataset. Furthermore, a previous study from the same population found no significant association between transient seropositive responses in cubs and infection in reproductively active females from the same social group [32]. Importantly, in the present study we found the same risk gradient for the detection of cub infection when we used the IFN-γ assay and detection of M. bovis by culture, both of which are unaffected by the immune status of the dam. It has also been suggested that the transmission of maternal antibody could protect cubs from future disease progression [33], although a more recent study of transient serological responses in cubs found

no evidence to support this hypothesis [32]. The risk gradient for cub excretion observed in the present study is also inconsistent with significant maternally derived immunological protection in cubs. The collective evidence therefore suggests that it is unlikely that colostrally derived antibody confounds the interpretation of our results.

Assigning parentage to cubs using genetic data was beyond the scope of our study. We assumed therefore, that all captured cubs were in potentially close contact with any females in their natal group which displayed evidence of reproductive activity. Alloparental care of young cubs, particularly by unsuccessful breeding adult females [15, 16], and an absence of competition among females following parturition [34], support our assumption.

Our results add to the evidence base underpinning disease control strategies in badgers, in particular, vaccination and/or culling. The success of a vaccination strategy against M. bovis in badgers depends on the efficacy of the vaccine in the individual, and delivery of vaccine to a sufficient proportion of susceptible badgers (by definition, prior to the acquisition of infection) to induce herd immunity, protecting the unvaccinated proportion of the population through reducing the quantity and frequency of mycobacterial excretion in the vaccinated population [35]. Studies using injectable BCG in badgers have shown a reduction in the severity of disease in individual badgers [36], and provided evidence of an indirect protective effect of vaccination to unvaccinated cubs [30]. In the present study, we found that the majority of cubs were not detected as infected. Their availability for capture in the present study indicates that it would be possible to trap them for parenteral vaccination, and as traps were baited with novel food items, it suggests that they may readily take a vaccine bait (should one become available). However, a proportion of cubs will inevitably acquire infection prior to initial emergence from the sett, but our data suggest that these cubs are most likely to be spatially clustered within social groups with infectious breeding females.

The extent to which badger culling may reduce the incidence of cattle herd breakdowns continues to be the subject of intense debate in the UK. Selective removal of infectious breeding females could theoretically contribute to reducing the incidence of cub infection in the badger population and could be combined with the removal of other infectious individuals. Selective removal of a limited number of individuals

within a social group is intuitively appealing as it may minimize the negative epidemiological effects associated with the perturbation of social structure in badger populations [37], although it is not known what proportion of a social group or which individuals (if any), can be removed before significant negative effects are incurred. Major constraints to selective removal are the current absence of a method for live testing in the field without recourse to anaesthesia, and a field test with greater sensitivity than the Brock TB SP test.

Predictive models have been used to evaluate the relative impacts of different intervention strategies aimed at controlling TB in badger and cattle populations [38, 39]. A previous model indicated that the release or removal of lactating female badgers in a population subjected to culling would have no effect on the subsequent prevalence of infection, suggesting that the selective removal of lactating females alone may not contribute positively to TB control in badger populations [40]. However, this model did not take into account the existence of a spatially explicit infection risk gradient as indicated by our findings.

In conclusion, our study has highlighted the epidemiological importance of infectious breeding female badgers, and suggests that simulation models used to evaluate potential control strategies could be improved by incorporating the consequent heterogeneity in individual infection risk. Furthermore, our findings provide further illustration of the importance of spatial organization in determining transmission risks in social animals.

ACKNOWLEDGEMENTS

DECLARATION OF INTEREST

None.

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