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Effects of culling on spatial associations of *Mycobacterium bovis* infections in badgers and cattle

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Summary

1. Bovine tuberculosis (TB), caused by *Mycobacterium bovis*, has serious consequences for Britain's cattle industry. European badgers (*Meles meles*) can transmit infection to cattle, and for many years the British government culled badgers in a series of attempts to reduce cattle infections.

2. We investigated the impact of badger culling on the spatial distribution of *M. bovis* infection in badger and cattle populations in replicated areas in England.

3. *M. bovis* infection was significantly clustered within badger populations, but clustering was reduced when culls were repeated across wide areas. A significant spatial association between *M. bovis* infections in badgers and cattle herds likewise declined across successive culls. These patterns are consistent with evidence that badgers are less territorial and range more widely in culled areas, allowing transmission to occur over greater distances.

4. Prior to culling, *M. bovis* infections were clustered within cattle populations. Where badger culling was localised, and in unculled areas just outside widespread culling areas, cattle infections became less spatially clustered as badger culling was repeated. This is consistent with expanded badger ranging observed in these areas.

5. In contrast, clustering of infection in cattle persisted over time on lands where badgers were repeatedly culled over wide areas. While this lack of a temporal trend must be interpreted with caution, it might reflect persistent infection within, and continued transmission between, cattle herds in areas where transmission from badgers to cattle had been reduced by badger culling. Continued spatial association of infections in cattle and badgers in such areas might partly reflect transmission from cattle.

6. *Synthesis and applications*: Our findings confirm that badger culling can prompt spatial spread of *M. bovis* infection, a phenomenon likely to undermine the utility of this approach as a disease control measure. Possible evidence of transmission from cattle, both to other cattle and to badgers, suggests that improved cattle controls might yield multiple benefits for TB management.

Key-words: badger, bovine tuberculosis, disease ecology, epidemiology, multihost, *Mycobacterium bovis*, perturbation, RBCT, wildlife disease, zoonosis

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Introduction

Bovine tuberculosis (TB), caused by *Mycobacterium bovis*, is a disease imposing substantial costs on Britain's cattle industry. Regular testing of cattle, with slaughter of those testing positive, has successfully controlled the infection across much of the developed world. However, control has not been achieved where wildlife populations have become persistently infected (Morris, Pfeiffer & Jackson 1994). In Britain, failure to control cattle TB has been linked to transmission of infection from badgers *Meles meles*, a wildlife species that thrives in landscapes where cattle are farmed (Neal & Cheeseman 1996). Badger culling therefore formed a component of British TB control policy for many years (Krebs *et al*. 1997).

Badger behaviour appears to play an important role in TB dynamics. At the high population densities which occur across most of Britain, badgers are both social and territorial (Kruuk 1989), and permanent transfer of animals between social groups is infrequent (Woodroffe, Macdonald & da Silva 1995). Correspondingly, *M. bovis* infections are highly clustered within affected badger populations (Olea-Popelka *et al*. 2003; Woodroffe *et al*. 2005c), and social groups experiencing high prevalence may occupy territories adjacent to those of uninfected groups (Cheeseman *et al*. 1981; Cheeseman *et al*. 1985). In undisturbed populations, these clusters of infection can remain stable for many years (Delahay *et al*. 2000).

M. bovis infections are likewise clustered within cattle populations (Woodroffe *et al*. 2005c). Clusters of cattle infection are spatially associated with those in badgers, and this association is particularly marked for animals sharing the same *M. bovis* strain type (Woodroffe *et al*. 2005c). These findings suggest that interspecific transmission influences the spatial distribution of *M. bovis* infection, but are not in themselves sufficient to determine whether badger-to-cattle or cattle-to-badger transmission is most important.

Field studies indicate that all transmission pathways (badger-to-badger, badger-to-cattle, cattle-to-badger and cattle-to-cattle) are important components of TB dynamics in Britain (Gilbert *et al*. 2005; Donnelly *et al*. 2006; Woodroffe *et al*. 2006b). However, badger culling is expected to have different effects on each of these pathways, with various possible outcomes for the clustering of infection within badger and cattle populations (Fig. 1), and for the spatial association between the two.

Within badger populations, culling profoundly disrupts social and territorial organization, leading badgers to range more widely (Woodroffe *et al*. 2006a). This is likely to increase contact rates between badgers, and may explain marked increases in *M. bovis* prevalence that have been detected in badger populations subjected to culling (Woodroffe *et al*. 2006b; R. Woodroffe, C.A. Donnelly, P. Gilks *et al.*, unpublished). Immigration of badgers into culled areas from neighbouring lands appears to contribute to this pattern (Woodroffe *et al*. 2006b).

If culling influences badger-to-badger transmission of *M. bovis* by disrupting territorial behaviour and expanding home range sizes, infections within the badger population would be expected to become less clustered in response to culling (Fig. 1c). This is because mixing, and hence transmission, is expected to occur between badgers originating at greater distances from one another, breaking up the clusters observed in undisturbed populations. Further, if the majority of *M. bovis* infections in cattle were acquired from badgers (Fig. 1b), any reduction in the degree of clustering within badger populations would be expected to cause a corresponding reduction in clustering within cattle populations (Fig. 1d). By contrast, badger culling would not be expected to influence infection clustering in cattle populations if most cattle infections were acquired from other cattle.

If culling-induced social disruption of badger populations influences *M. bovis* transmission as proposed, culling would be expected to reduce the spatial association observed between infections in cattle and badgers, irrespective of whether this association was generated mainly by badger-to-cattle or by cattle-to-badger transmission. This is because the expanded

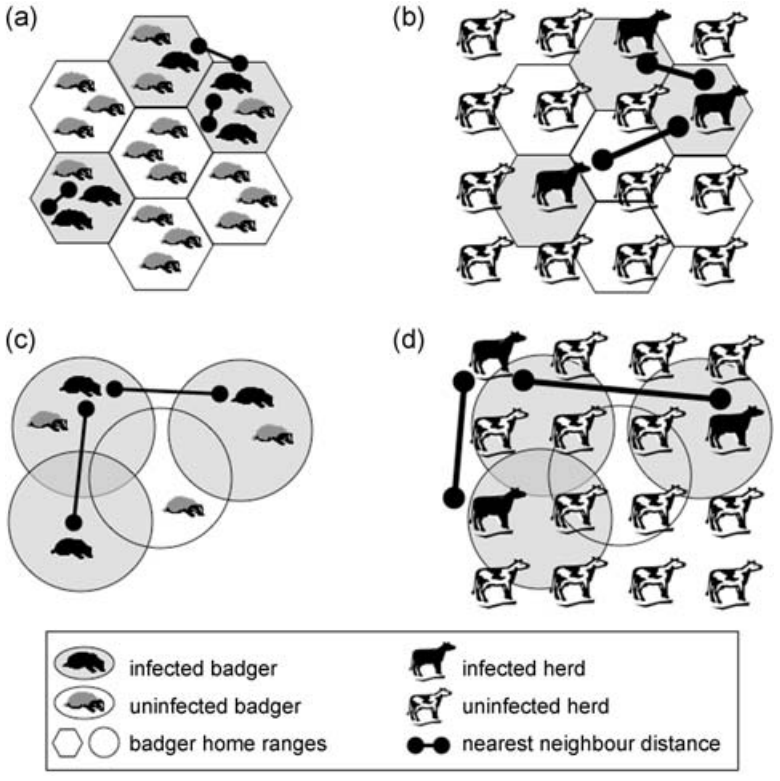

Fig. 1. Predicted effects of badger culling on the spatial distribution of *M. bovis* infection. In undisturbed populations, badger territoriality limits disease spread among badgers (a) and to cattle (b), constraining the spatial scale of infection clustering. Proactive culling lowered badger density and expanded their ranging; this is predicted to reduce clustering of infection in badgers (c). If badgers remained a major source of cattle infections inside proactive areas, reduced clustering of infection in badgers would be mirrored in cattle (d).

ranging behaviour observed in badgers in culled areas (Woodroffe *et al*. 2006a) is likely to allow transmission between the two host species over greater distances. In addition, as culling reduces badger density, it is expected to lower the proportion of cattle infections caused by badgers, and hence should reduce the spatial association between infections in the two hosts.

These predictions indicate that describing the impact of badger culling on the spatial distribution of *M. bovis* infection will be valuable in designing future strategies for cattle TB control. First, such a description will test the hypothesis that alterations to badger spatial organization can influence the geographical distribution of *M. bovis* infection. This could explain the capacity of badger culling to increase infection rates in cattle where culling is localised in small areas, and on unculled land adjoining widespread culling areas (Donnelly *et al*. 2003; Donnelly *et al*. 2006; Donnelly *et al*. 2007), and could therefore help to determine whether other culling methods may be devised to avoid these detrimental effects. Second, comparing the effects of badger culling on infection clustering in badgers and cattle might potentially shed light on the importance of badger-to-cattle, cattle-to-badger and cattle-to-cattle transmission. This would help to determine the potential value of future TB management strategies targeted at badgers and cattle.

We investigated the effects of badger culling on the spatial distribution of *M. bovis* infection in badgers and cattle, using data from the Randomised Badger Culling Trial (RBCT), a large-scale field study of badger culling as a strategy to control cattle TB in high-risk areas of England (Donnelly *et al*. 2003; Donnelly *et al*. 2006). Previous analyses (Woodroffe *et al*. 2005c) considered data collected at the start of the RBCT, when badger populations were comparatively undisturbed by culling. Here, analyses are expanded to consider the effects of repeated culling conducted within the same trial areas. Specifically, we predicted that repeated badger culling would reduce the clustering of *M. bovis* infections within both the badger and cattle populations, and would reduce the spatial association between infections in badgers and cattle.

Materials and methods

STUDY AREAS

Details on the design and implementation of the RBCT are provided elsewhere (Bourne *et al*. 1998; Donnelly *et al*. 2003; Donnelly *et al*. 2006) but, in summary, the study was designed to compare TB incidence in cattle under three conditions of badger culling: widespread 'proactive' culling (which aimed to maintain low badger densities across large areas for the duration of the RBCT); localised 'reactive' culling (which aimed to cull only those badgers spatially associated with farms that had experienced recent TB outbreaks in cattle); and no culling (an experimental control). Each of these treatments was replicated 10 times in trial areas of approximately 100 km^2 each, to give a total of 30 trial areas (3000 km^2) grouped into 10 'triplets'. Most of the data presented here (including all the badger data) relate to the 10 'proactive culling' areas of the RBCT, and were collected in 1998–2005. Reactive culling occurred in nine areas in 1999–2003. Trial area locations and cull dates are shown in the Supplementary Material.

BADGER CULLING

Within each proactive trial area, an initial cull was carried out simultaneously across all land to which landholders granted access (approximately 70% of the total, Donnelly *et al*. 2007). Follow-up proactive culls were repeated approximately annually (with longer delays incurred in 2001 due to a nationwide epidemic of foot-and-mouth disease (FMD); details in Supplementary Material). All 10 proactive areas received the first four culls, four received five culls, two received six culls, and one received seven culls (see Supplementary Material for cull dates). Nine reactive areas received between 1 and 4 years of reactive culling; culling had not yet been commenced in the tenth area when evidence of the detrimental effects of reactive culling became apparent (Donnelly *et al*. 2003) and the reactive treatment was suspended by Ministers.

Badgers were captured in cage traps, placed mostly at setts (badger dens), and were dispatched by shooting. Independent audits deemed dispatch 'humane' (Kirkwood 2000), and confinement in the trap caused no detectable injury in the majority of badgers (Woodroffe *et al*. 2005b). No culling was undertaken in February – April to avoid killing the mothers of dependent cubs confined to the sett (Woodroffe *et al*. 2005a). Badger capture locations were recorded in the field on a 100 m grid.

DIAGNOSTIC PROCEDURES FOR BADGERS

All badger carcasses were chilled and then subjected to necropsy, usually within 72 h of dispatch. After recording basic data on age, sex, and body size, a standard set of lymph node samples (retropharyngeal, bronchial and mediastinal) were collected, as well as samples of any lesions suggestive of TB. These tissues were then cultured for evidence of *M. bovis* infection. Badgers were considered infected if *M. bovis* was cultured, or if acid-fast bacteria were detected by Ziehl Neelsen staining of sections of lesioned tissue. Isolates of *M. bovis* cultured from trial badgers were strain typed by spacer oligonucleotide typing ('spoligotyping', Kamerbeek *et al*. 1997). Spoligotype data were available for 971 (99%) of the 982 proactively culled culture-positive adult badgers.

Around 10% of carcasses were stored (almost always frozen) for > 7 days before necropsy. Since such storage appears to reduce the probability of detecting infection

in badgers (Woodroffe *et al*. 2006b), these carcasses were excluded from primary analyses. Alternative analyses, including these animals, are provided in Supplementary Material. Analyses likewise excluded badger cubs, since these show markedly lower *M. bovis* prevalence than do adults (Woodroffe *et al*. 2006b).

CATTLE TB DATA

Data on *M. bovis* infections in cattle were collected using routine veterinary surveillance. In high TB-risk areas (including all trial areas), cattle are subjected to annual tuberculin testing; test-positive animals are compulsorily slaughtered and subjected to necropsy. If lesions characteristic of TB are identified, the herd is considered 'lesion-positive'. Infection in the herd is considered 'confirmed' if lesions are identified, or if *M. bovis* infection is detected by culture of necropsy samples. Within trial areas, policy was to culture tissue samples from all compulsorily slaughtered cattle. Outside trial areas, samples were routinely cultured from up to 3 slaughtered cattle from herds with multiple visibly lesioned animals, up to 5 slaughtered cattle from herds with one bovine with a single lesion, or up to 10 slaughtered cattle if no lesions were detected. In addition, the Meat Hygiene Service inspects all cattle sent for slaughter and, if suspected TB lesions are identified, samples are collected and cultured; slaughterhouse cases trigger a test in the herd of origin. Isolates of *M. bovis* cultured from cattle in the trial areas were subjected to spoligotyping as for badgers.

Following Woodroffe *et al*. (2005c), we considered all herds showing evidence of infection, whether from tuberculin testing, culture, or detection of lesions to be 'TB-affected'. We further distinguished TB-affected herds with and without lesions indicative of TB. Alternative analyses considered herds to be 'TB-affected' only if infection was confirmed (from lesions or culture); this is because badger culling was found to influence the incidence of confirmed but not unconfirmed TB outbreaks in cattle (Donnelly *et al*. 2007). Following Woodroffe *et al*. (2005c), herd locations inside trial areas were taken from the RBCT database, and those from nearby areas (which were only partially covered by the RBCT database) were taken from the State Veterinary Service's VetNet database.

Our primary analyses of the spatial distribution of TB-affected cattle concerned the 12 months prior to (the last day of) each badger cull (the 'pre-cull period'). A herd was considered 'TB-affected' in a particular period if evidence of infection was detected at any time during that period (hence, analyses were not restricted to newly detected outbreaks). Since all cattle inside RBCT areas were required to have annual tuberculin tests, this 12-month period should represent complete testing of the herds in each trial area. However, very little routine TB testing was undertaken during the FMD epidemic that occurred from 20 February to 28 November 2001 (Cox *et al*. 2005). Hence, to ensure that data on the distribution of infection in badgers were compared with data on tests of all cattle herds, we extended the comparison periods to include 12 months of routine testing. A small number of test results from the FMD period were also included but were too few to influence the outcome of analyses. As an example, for a badger culled on 18 October 2002, the comparison period of cattle testing prior to culling was 9 January 2001–18 October 2002.

Following Woodroffe *et al*. (2005c), we also analysed the spatial distribution of infection detected in cattle during the 12 months following (the last day of) each cull (the 'post-cull period'); these results are presented in Supplementary Material. However, we considered analyses from the pre-cull period more informative, since a high proportion of the cattle tested during this period would have had opportunities for contact with the badgers subsequently culled. This is particularly likely for the period preceding the initial culls since, prior to RBCT culling, badgers' longevity and naturally low dispersal rates (Woodroffe *et al*. 1995) would mean that most individuals (particularly the adults included in these analyses) would have been culled in the areas which they had inhabited during the preceding year. In contrast, movement rates of cattle are comparatively high (Gilbert *et al*. 2005) so that, during each post-cull period, an increasing proportion of the cattle population would have been bought in since the last cull and would be unlikely to have contacted the culled badgers.

Spoligotype data were available for 95·7% of 9398 culture-positive cattle, and for 98·4% of culture-positive herds, that were tested within 10 km of proactive badger capture locations, in the 12 months before or after proactive culling.

STATISTICAL ANALYSES

Our analyses of spatial associations of infection were based on nearest neighbour distances from badgers to other badgers, from cattle herds to other cattle herds, and from badgers to cattle herds (calculated using ArcGIS version 9·0, ESRI, Redlands, CA). Statistical analyses were carried out in SAS (SAS Institute, Cary, NC). All analyses involving badger locations include data from proactive culling areas only, since these are the only places where badgers were sampled systematically. In contrast, analyses of spatial associations within cattle populations could be analysed for all RBCT treatments.

We used regression models to test whether infected badgers were, on average, closer to other infected badgers than were uninfected badgers; such evidence would indicate spatial clustering of infections. This analysis approach was used in preference to the methods used by Woodroffe *et al*. (2005c), because it was expected to give comparable results despite changes in badger density. The outcome variable for these models was the natural logarithm of the distance from the index badger's capture location to the capture location of the

nearest infected badger (Fig. 1a). Predictor variables were the infection status of the index badger (infected or uninfected), the triplet in which the index badger was captured, and cull number (a categorical variable defined to be 1 for the initial proactive cull, 2 for the first follow-up cull and so on (maximum cull number = 7)). Primary analyses also included an interaction term for infection status*cull number, to determine whether the pattern of clustering differed across successive culls. A triplet*cull number interaction was also included, as was the three-way infection status*triplet*cull number interaction. The three-way interaction was treated as a random effect which would increase the variance associated with parameter estimates, accounting for any overdispersion in the data. When calculating the confidence intervals around these results, we used *t*-values (instead of *z*-values) to allow for the number of degrees of freedom associated with the standard error of each estimate.

We used the outputs from these models to test for a linear trend in clustering on successive culls, fitting cull number as a continuous variable using weighted least squares. As these linear trends were on a log scale, the actual (exponentiated) trends were proportional; a negative slope indicated a reduction in clustering. We tested the robustness of these linear trends by excluding data from each cull number in turn. We also investigated the overall degree of clustering (on all culls combined) by excluding the infection status*cull number interaction and examining the main effect of infection status.

When the cumulative proportion of badger-to-infected badger distances was plotted against the square of the distance (a proxy for the area searched to locate the nearest infected badger), an inflection point was observed at around 1·25 km for both infected and uninfected index badgers (Fig. 2a). This change from a steeper slope to a shallower slope, which occurs around the 90th percentile (Fig. 2a), indicates stronger clustering at distances up to 1·25 km. To characterise this clustering in a regression model with a single slope, we excluded the longest 10% of distances (within each cull and TB status) from all analyses. Alternative results including these distances are presented in Supplementary Material.

Regression models similar to those used for badger-to-badger distances were also fitted to the distance from an index cattle herd to the nearest TB-affected cattle herd. Since the practice of testing contiguous herds when infection was detected in cattle could artificially generate the appearance of clustering, we repeated cattle-to-cattle analyses excluding the results of contiguous testing. Likewise, we repeated key analyses accounting for possible effects of including some test results in more than one 12-month period (which occurred occasionally when successive proactive culls were conducted less than a year apart); methods are detailed in Supplementary Material.

Results of cattle-to-cattle analyses from inside the proactive areas were also compared with those from

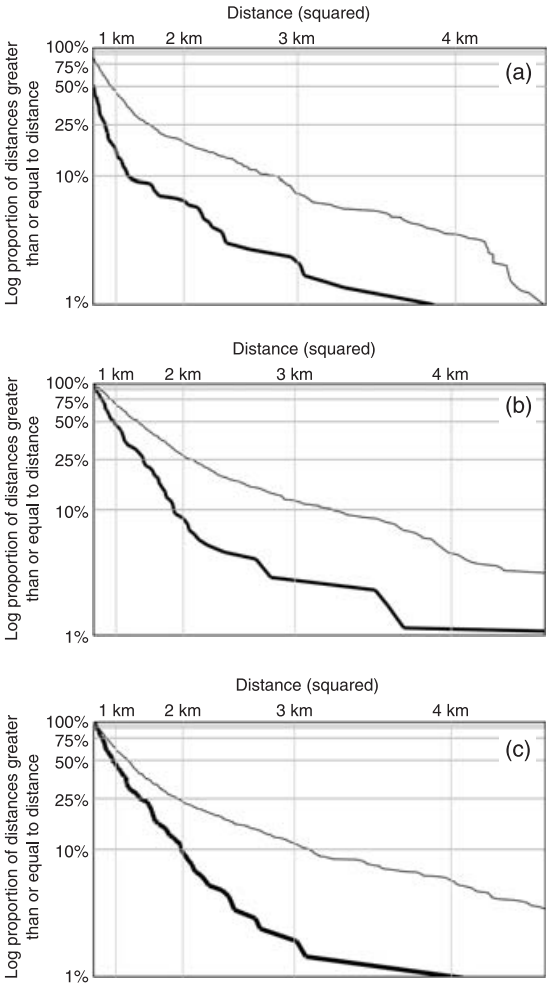

Fig. 2. Spatial relationships between *M. bovis* infections from initial proactive culls. (a) The distance between neighbouring pairs of badgers (plotted on the scale of distance squared, as a proxy for the area searched to locate the nearest neighbour), plotted against the proportion of distances greater than or equal to that distance (on a log scale). The thick line indicates distances from infected badgers to other infected badgers, and the narrow line indicates distances from uninfected badgers to infected badgers. (b) and (c) The equivalent information for cattle-to-cattle and badger-to-cattle distances, respectively, measured during the pre-cull period.

herds on land ≤ 2 km outside the proactive areas, and from reactive and no-culling trial areas, to further explore potential effects of badger culling on clustering of TB cases in cattle. Since (unlike proactive culling) reactive culling was not repeated systematically across trial areas, analyses considered the 12 months preceding the first reactive cull in each triplet, and successive 12 months periods thereafter, ending with the 12-month period including the date that reactive culling was suspended (4 November 2003). This gave a maximum of four 12-month periods per triplet. Analysis periods for no-culling areas were the same as for proactive areas.

A similar analytical approach was adopted in comparing distances from an index (proactively culled) badger to the nearest TB-affected cattle herd. For these analyses, data were summarised for badgers trapped at

the same location (with the same distance to the nearest infected herd), to avoid spurious precision in the resulting statistics that could be caused if similar badgers trapped at a single location were treated as producing independent observations. However, a single location could contribute data both as *M. bovis* infected (if one or more infected badgers were trapped there) and as uninfected (if one or more uninfected badgers were trapped there).

In addition to these primary analyses, we also analysed distances relative to badgers or cattle with lesions suggestive of TB disease. Such animals are widely considered more likely to be infectious than are infected animals without lesions, and might therefore show particularly close spatial associations with other infected animals.

We also investigated clustering of *M. bovis* spoligotypes in proactive areas. To do this we calculated, for each infected badger, the ratio of the distance to the nearest badger of the same spoligotype, to the distance to the nearest badger of a different spoligotype (adding 50 m to all distances to avoid infinite ratios associated with distances of zero). These ratios were averaged for each trial area and each cull, and then analysed using normal regression models with cull number as a categorical variable, weighted by the number of ratios contributing to each average ratio. Similar analyses investigated spatial associations of *M. bovis* spoligotypes between cattle herds, and between badgers and cattle herds. For analyses of badger-to-cattle distances, each badger-capture location and each herd contributed one observation for each spoligotype detected therein.

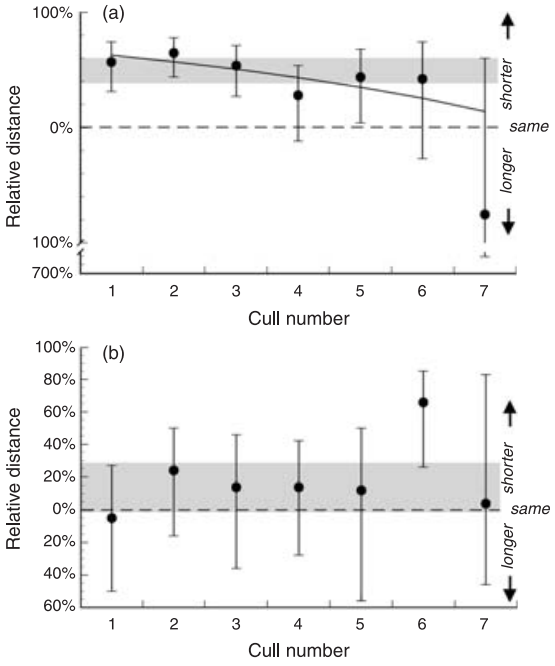

Fig. 3. Clustering of *M. bovis* infections in badgers on successive proactive culls. (a) The percentage difference between infected and uninfected badgers in the distance to the nearest infected badger, with shorter relative distances indicating stronger clustering. Results are derived from regression models including adult badgers only; error bars denote 95% confidence intervals. The grey shading shows the 95% confidence interval around the estimate for all culls combined and the solid line shows a significant linear (on a log scale) trend across culls. (b) Equivalent data for distances to the nearest infected badger from other infected badgers with and without lesions.

Results

CLUSTERING OF INFECTION WITHIN BADGER POPULATIONS

The distance to the nearest infected badger was, on average, 49% shorter (95% confidence interval (CI) 38–59% shorter) from infected than from uninfected badgers, indicating significant clustering of *M. bovis* infections within badger populations. This difference was consistent across a range of spatial scales; for example, Fig. 2a shows that about 40% of distances from an uninfected badger to the nearest infected badger exceeded 1 km, whereas the corresponding percentage for infected badgers was about 20%.

The extent of infection clustering within the badger population varied between culls (Fig. 3a). The average percentage difference between infected and uninfected badgers in the distance to the nearest infected badger gives a measure of clustering which can be compared across culls, with differences of 0% indicating no clustering. This measure declined significantly (P = 0·004) on successive culls, with the linear trend (on a log scale) equating to a proportional decline of 14·9% (95% CI 4·5–26·2% decline) with each cull (Fig. 3a). The trend remained significant when data from each cull number were omitted from the analysis in turn, indicating a robust effect (details in Supplementary Material). The negative trend indicates that infections were becoming less clustered on successive culls.

There was a borderline nonsignificant trend suggesting that lesioned badgers might have been closer to infected badgers than were those that were infected but lacked detectable lesions (distances from lesioned badgers were 15% shorter, 95% CI 1% longer to 29% shorter; Fig. 3b). This pattern of spatial association did not change across successive culls (Fig. 3b).

CLUSTERING OF INFECTION WITHIN CATTLE POPULATIONS

Inside proactive areas, the distance to the nearest TB-affected cattle herd was, on average, 16% shorter (95% CI 8–23% shorter; Fig. 4a) from affected than from unaffected herds, indicating significant clustering of *M. bovis* infections between cattle herds. Results were similar for the post-cull period, and for analyses excluding contiguous testing (see Supplementary Material). Primary analyses revealed no trend in clustering over successive pre-cull periods (slope –0·12%, 95% CI –2·41% to +2·11%). Results were similar when standard errors were adjusted to account for inclusion

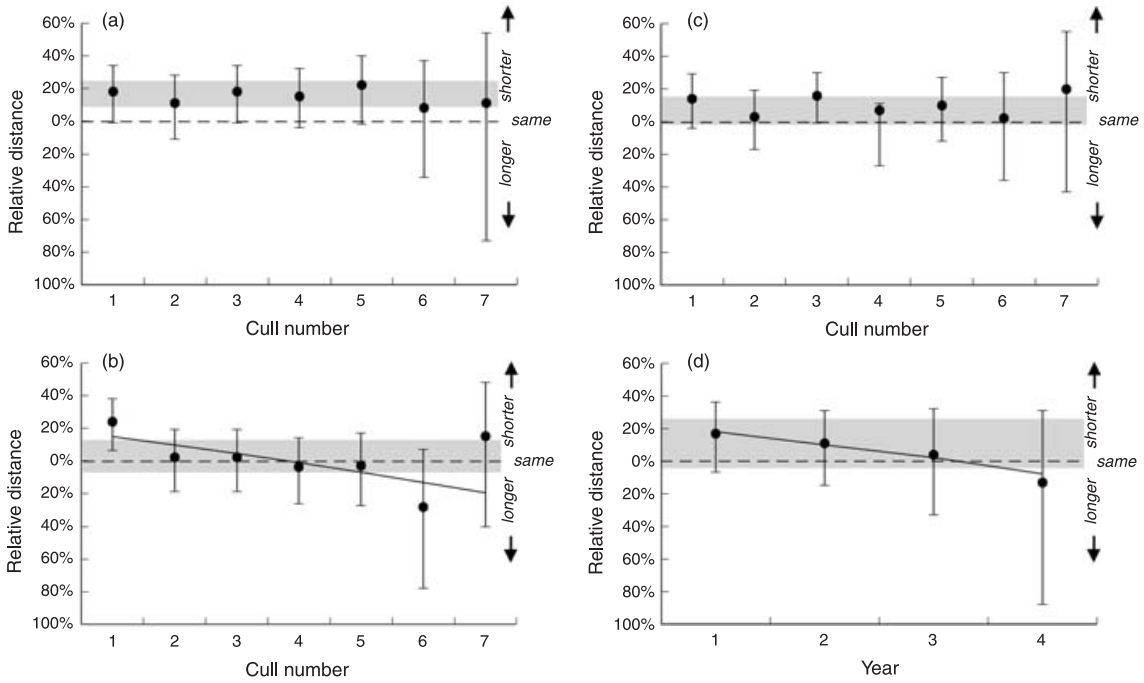

Fig. 4. Clustering of *M. bovis* infections in cattle. Graphs show the percentage difference between TB-affected and unaffected herds in the distance to the nearest affected herd, for (a) inside and (b) ≤ 2 km outside proactive areas, and for (c) survey-only, and (d) reactive areas. Shorter relative distances indicate stronger clustering. Error bars denote 95% confidence intervals, grey shading shows the 95% confidence interval around the estimate for all time periods combined, and solid lines show significant linear (on a log scale) trends across time periods.

of some test results in more than one time period (details in Supplementary Material).

In contrast, the clustering of infection within cattle populations declined over time both ≤ 2 km outside proactive areas (slope –5·79%, 95% CI –1·47% to –10·31%, $P = 0{\cdot}008$; Fig. 4b), and inside reactive areas (slope –9·64%, 95% CI –7·24% to –12·10%, $P < 0{\cdot}001$; Fig. 4d). No such trend was detected in no-culling areas (slope –1·53%, 95% CI –5·77% to +2·53%; Fig. 4c).

There was no evidence that lesioned herds were more closely associated with TB-affected herds than were affected herds without lesions (details in Supplementary Material).

ASSOCIATIONS BETWEEN INFECTIONS IN BADGERS AND CATTLE

Inside proactive areas, *M. bovis* infections in cattle were spatially associated with those in badgers. The distance to the nearest TB-affected cattle herd was, on average, 14% shorter (95% CI 9–19% shorter) for infected than for uninfected badgers (Fig. 5a).

The degree of spatial association between infections in badgers and cattle herds varied between culls. For the pre-cull period, this measure declined significantly ($P = 0{\cdot}005$) on successive culls, with the linear trend (on a log scale) equating to a proportional decline of 3·67% (95% CI 1·12–6·28%) with each cull (Fig. 5a). The relationship remained significant when cull numbers 2–7 were in turn excluded from the analysis, but became nonsignificant ($P = 0{\cdot}139$) when the initial cull was excluded (details in Supplementary Material), illustrating the important difference between the initial and all subsequent culls. The negative relationship indicates that cattle infections in the pre-cull periods were becoming less spatially associated with badger infections on successive culls.

There was no evidence to suggest that badgers with lesions suggestive of TB were particularly closely associated with infected cattle: lesioned badgers were no closer to TB-affected cattle herds than were unlesioned badgers (Fig. 5b), although statistical power was reduced due to small sample sizes. By contrast, *M. bovis* infections in badgers may have been particularly closely associated with lesioned cattle. The distances from infected badgers to lesioned herds were significantly shorter than those from uninfected badgers to such herds, by proportions as great as, or greater than, those observed for all infected herds (Fig. 5c). This association declined on successive culls, equivalent to a 3·62% (95% CI 0·23–7·14%) reduction on each cull.

ASSOCIATIONS BETWEEN STRAIN TYPES OF *M. BOVIS*

Distances from infected badgers to other badgers infected with the same *M. bovis* spoligotype were consistently and significantly shorter than distances to badgers infected with different spoligotypes (Fig. 6a), indicating marked clustering. There was similar (and equally significant) evidence of spoligotype clustering between cattle herds (Fig. 6b), and of spatial association of *M. bovis* spoligotypes in cattle and badgers (Fig. 6c).

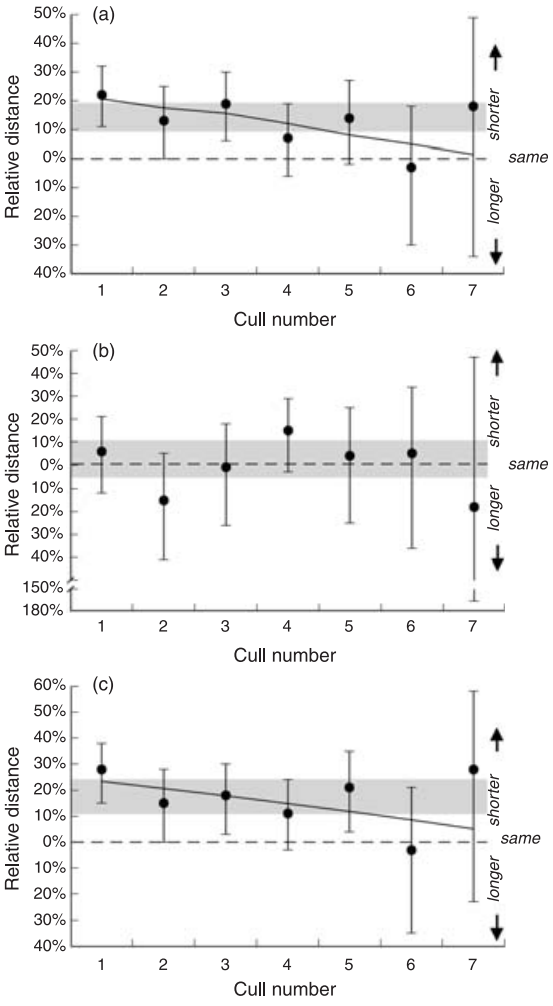

Fig. 5. Spatial association of *M. bovis* infections in badgers and cattle in proactive areas. (a) The percentage difference between infected and uninfected badgers in the distance to the nearest TB-affected herd, with shorter relative distances indicating stronger spatial association. Results are derived from regression models including adult badgers only; error bars denote 95% confidence intervals. The grey shading shows the 95% confidence interval around the estimate for all culls combined and the solid line shows a significant linear (on a log scale) trend across culls. (b) The equivalent data for distances to the nearest TB-affected herd from infected badgers with and without lesions. (c) The equivalent data for distances to the nearest infected badger from TB-affected herds with and without lesions.

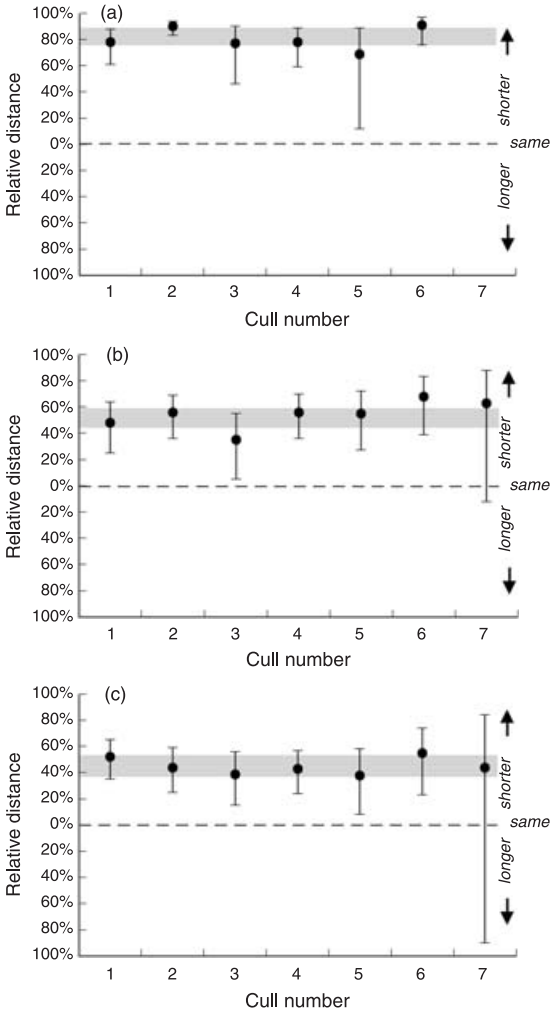

Fig. 6. Spatial association of *M. bovis* spoligotypes. (a) The percentage difference between the distances from an infected badger to the nearest badger with the same, and a different, spoligotype: shorter distances indicate closer spatial associations. There are no data for cull 7 because all infected badgers captured on this (single) cull had the same spoligotype. (b) and (c) Equivalent data for cattle-to-cattle and cattle-to-badger distances. Error bars denote 95% confidence intervals around estimates for each cull number, and grey shading shows the 95% confidence interval around the estimate for all culls.

However, this spoligotype clustering did not increase or decrease across successive culls in either host species.

Discussion

Our findings provide valuable insights into the dynamics of *M. bovis* in British agricultural landscapes, and into the effects of badger culling on *M. bovis* epidemiology.

CLUSTERING OF INFECTION WITHIN BADGER POPULATIONS

Within proactive areas, *M. bovis* infection was clustered within badger populations, on a scale of around 1 km; this has been recorded in previous studies (Olea-Popelka *et al*. 2003; Woodroffe *et al*. 2005c).

As predicted, the degree of infection clustering within badger populations declined on successive proactive culls. This pattern is consistent with the observation that badger ranging behaviour expands in response to culling (Woodroffe *et al*. 2006a); such a behavioural change would allow transmission to occur between badgers originating at greater distances from one another. The effect is unlikely to be an artefact caused by reduction in badger density, since our analysis method (comparing the distance from infected and uninfected badgers to the nearest infected badger) accounted for changing badger-to-badger distances on successive culls, and so was robust to changing badger

density. The results therefore suggest that the spatial scale of badger-to-badger transmission increased in response to repeated culling.

ASSOCIATIONS BETWEEN INFECTIONS IN BADGERS AND CATTLE

M. bovis infections in badgers and cattle were likewise spatially associated inside proactive areas; this is consistent with findings from previous studies (Olea-Popelka *et al*. 2005; Woodroffe *et al*. 2005c).

The spatial association between infections in badgers and cattle herds declined on successive proactive culls (Fig. 5a). As described earlier, this pattern is to be expected for two reasons. First, badgers' expanded ranging behaviour in culled areas would allow infectious contact to occur with cattle at greater distances from the badgers' origins, whether such contact resulted in badger-to-cattle or cattle-to-badger transmission. Second, as badger culling is known to have reduced badger-to-cattle transmission of infection inside proactive culling areas (Donnelly *et al*. 2006; Donnelly *et al*. 2007), a smaller proportion of cattle infections would be caused by badgers, reducing spatial association between infections in the two hosts.

CLUSTERING OF INFECTION WITHIN CATTLE POPULATIONS

Inside proactive areas, there was a consistent pattern indicating clustering of *M. bovis* infection between cattle herds; however, this pattern did not change across successive culls (Fig. 4a). Since *M. bovis* infections in the badger populations became less clustered on successive proactive culls, and spatial associations between badgers and cattle likewise declined, it is somewhat surprising that the degree of clustering within the cattle population showed no evidence of a change over the same time period.

In contrast with the pattern observed inside proactive areas, clustering of infections in cattle did decline over time on unculled land just outside proactive areas, and also in reactive areas. Since badgers were not culled systematically across these areas, it is not known whether the spatial distribution of *M. bovis* infection in badgers changed in response to culling as it did inside proactive areas. However, badger ranging behaviour was expanded in these areas (Woodroffe *et al*. 2006a), and it is therefore likely that transmission of infection was facilitated across wider areas. Such spatially expanded badger-to-cattle transmission offers a plausible explanation for the reductions in infection clustering that we observed in cattle.

These results from reactive areas, and from unculled land just outside proactive areas, show that badger culling had the capacity to alter the spatial distribution of infection in cattle, on a timescale detectable within the course of our study. Since this is the case, it is, perhaps, surprising that no similar temporal trend was detected inside proactive areas. While caution must be exercised in interpreting the absence of a detected trend, if the observed pattern is correct it would suggest that the changing geographical distribution of infection in badgers inside proactive areas was insufficient to alter the distribution of infection in local cattle, over the time frames analysed. The most likely explanation for this pattern is that a comparatively small proportion of cattle infections inside proactive areas might have been caused by badgers. This is to be expected, since reducing badger-to-cattle transmission was the purpose of proactive culling (Krebs *et al*. 1997; Bourne *et al*. 1998), and since badger densities were substantially reduced in proactive areas (Woodroffe *et al*., in press). It should be noted that this conclusion applies only to areas in which badger density has been substantially reduced by widespread culling, not to unculled areas. A higher rate of badger-to-cattle transmission would be expected where badgers are not culled, and therefore remain at natural population densities.

If the spatial distribution of *M. bovis* infection within cattle populations inside proactive areas really was consistent throughout the course of the RBCT, despite changes in the corresponding distribution in badgers, two questions arise. First, what was the mechanism that maintained clustering between cattle herds? If the arguments outlined above are correct, it appears unlikely that infection clusters in cattle were maintained by spillover of infection from corresponding clusters in badgers. One possibility is that infection spread between neighbouring herds, either through direct contact or through local trading of cattle. It is also possible that persistent infection within cattle herds (which may occur despite repeated testing due to the imperfect sensitivity of the tuberculin test, Morrison *et al*. 2000), could help to maintain infection clusters.

A second question raised by the apparent persistence of infection clusters in cattle is why infections in cattle and badgers remained spatially associated (this association was significant up to cull 3, and retained a strong trend up to cull 5; Fig. 5a). If, as hypothesised above, proactive culling substantially reduced badger-to-cattle transmission, the spatial association between infections in the two species might have been maintained in part by continued cattle-to-badger transmission. This would be consistent with the evidence of widespread cattle-to-badger transmission in the same areas associated with the 2001 FMD epidemic (Woodroffe *et al*. 2006b), and may further emphasise the importance of this transmission route in *M. bovis* dynamics.

ASSOCIATIONS WITH LESIONED ANIMALS

Neither infected badgers nor infected cattle showed significantly greater spatial association with lesioned members of the same species, suggesting that the detection of lesions may be an unreliable indicator of infectiousness. This is consistent with observations of infectiousness in the absence of lesions in cattle

(McCorry *et al*. 2005), and the generally mild pathology observed in badger populations which nevertheless show high prevalence of infection (H.E. Jenkins, W.I. Morrison, D.R. Cox *et al*., unpublished). Although – as in previous analyses (Woodroffe *et al*. 2005c) – infected badgers were particularly closely associated with lesioned cattle, this may indicate greater association with confirmed outbreaks (Donnelly *et al*. 2007) rather than greater infectiousness of cattle with lesions.

ASSOCIATIONS BETWEEN STRAIN TYPES OF *M. BOVIS*

As in previous analyses (Woodroffe *et al*. 2005c), clustering of infection was particularly marked for animals sharing the same *M. bovis* spoligotype. This spoligotype clustering did not change on successive culls. This pattern is unsurprising since *M. bovis* spoligotypes in Britain occur as clones which are geographically localised on a scale much larger than the size of the trial areas studied here (Smith *et al*. 2003). A single spoligotype tends to dominate in each geographical region (Smith *et al*. 2003), so the power to detect changes in spoligotype clustering would have been limited.

IMPLICATIONS FOR MANAGEMENT

Our findings have potentially important implications for the design of future strategies to control cattle TB. First, they confirm that badger culling can alter the spatial distribution of *M. bovis* infection in both badgers and cattle. This is consistent with previous results (Woodroffe *et al*. 2006b), and provides a mechanism that helps to explain the elevated cattle TB incidence recorded on un-culled land adjoining proactively culled areas (Donnelly *et al*. 2006; Donnelly *et al*. 2007), and in regions where culling is restricted to localised areas (Donnelly *et al*. 2003). Hence, our results confirm that – in the absence of barriers to badger movement – TB control strategies based on badger culling are likely to risk detrimental effects for cattle TB incidence on neighbouring lands. Careful consideration is therefore needed to determine whether such strategies will have overall benefits for TB control.

A second management implication of our work relates to our finding of patterns consistent with continued transmission of *M. bovis* infection from cattle, both to other cattle and to badgers. This suggests that improved cattle-based controls could contribute to TB management, not only by reducing cattle-to-cattle transmission, but also potentially by limiting cattle-to-badger transmission, with the latter likely to have long-term benefits for reducing future re-infection of cattle.

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Supplementary material

The following supplementary material is available for this article.

Appendix S1. Detailed methods and results of the randomised badger culling trial.

Fig. S1. Locations of proactive culling, reactive culling and no culling areas of the RBCT.

Table S1. Dates of proactive culls by triplet and cull number.

Table S2. Approximate dates of reactive culling, by triplet and badger year.

Table S3. Numbers of badgers included in analyses.

Table S4. Numbers of cattle herds included in analyses presented from proactive areas.

Table S5. Clustering of *M. bovis* infection within badger populations.

Table S6. Median distances to the nearest infected badger or TB-affected herd, measured from badgers and herds with and without evidence of infection, in proactive areas.

Table S7. Robustness of the linear relationship between cull number and *M. bovis* clustering in badgers.

Table S8. Effect of lesions on *M. bovis* clustering within badger populations.

Table S9. Clustering of infection within cattle populations in proactive areas.

Table S10. Clustering of infection within cattle populations in proactive areas, excluding contiguous tests.

Table S11. Analysis of the linear relationship between cull number and *M. bovis* clustering in cattle inside proactive areas.

Table S12. Clustering of *M. bovis* infections in cattle in proactive areas, accounting for overlapping observation periods.

Table S13. Effect of lesions on clustering of infections in cattle populations in proactive areas.

Table S14. Clustering of *M. bovis* infection within cattle populations in survey-only areas.

Table S15. Clustering of *M. bovis* infection within cattle populations ≤ 2 km outside proactive areas.

Table S16. Clustering of *M. bovis* infection within cattle populations in reactive areas.

Table S17. Spatial association of *M. bovis* infection in cattle and badgers in proactive areas.

Table S18. Robustness of the linear relationship between cull number and spatial association of *M. bovis* infections in cattle and badgers.

Table S19. Spatial association between lesioned badgers in proactive areas and infected cattle.

Table S20. Spatial association of lesioned cattle and infected badgers in proactive areas.

Table S21. Spatial associations of *M. bovis* spoligoypes within and between badger and cattle populations, on successive proactive badger culls.

Table S22. Median distances to the nearest badger or herd with the same, or a different, spoligotype as the index animal.

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