Successive use of shared space by badgers and cattle: implications for *Mycobacterium bovis* transmission

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Summary

Managing infectious disease demands understanding pathogen transmission. In Britain, transmission of *Mycobacterium bovis* from badgers (*Meles meles*) to cattle hinders the control of bovine tuberculosis (TB), but the mechanism of such transmission is uncertain. As badgers and cattle seldom interact directly, transmission might occur in their shared environment through contact with contamination such as faeces, urine, and saliva. We used concurrent GPS-collar tracking of badgers and cattle at four sites in Cornwall, southwest Britain, to test whether each species used locations previously occupied by the other species, within the survival time of *M. bovis* bacteria. Although analyses of the same dataset showed that badgers avoided cattle, we found no evidence that this avoidance persisted over time: neither GPS-collared badgers or cattle avoided space which had been occupied by the other species in the preceding 36h. Defining a contact event as an animal being located <5m from space occupied by the other species within the previous 36h, we estimated that a herd of 176 cattle (mean herd size in our study areas) would contact badgers at least 6.0 times during an average 24h period. Similarly, we estimated that a social group of 3.5 badgers (mean group size in our study areas) would contact cattle at least 0.76 times during an average night. Such frequent successive use of the same shared space, within the survival time of *M. bovis* bacteria, could potentially facilitate *M. bovis* transmission via the environment.

Introduction

Understanding pathogen transmission is important for managing infectious disease. Assumptions about transmission mechanisms can profoundly affect predicted dynamics, and hence chosen control measures (Joh *et al.* 2009, Breban 2013). For example, if avian influenza is assumed to be transmitted among waterbirds via a faecal-oral route, epidemics are predicted to fade out rapidly whereas, if infection can also be acquired from contaminated water, longer epidemics and secondary outbreaks are predicted, so management efforts may need to be prolonged (Rohani *et al.* 2009).

In Britain, a limited understanding of transmission mechanisms hinders efforts to control bovine tuberculosis (TB, caused by *Mycobacterium bovis*). From near-eradication in the 1970s, the disease re-emerged in British cattle despite intensive control efforts (Pritchard 1988, Defra 2014). Transmission from badgers (*Meles meles*) contributes to the maintenance of the disease in cattle (Donnelly & Nouvellet 2013, Brooks-Pollock & Wood 2015, Crispell *et al.* 2019). However, the mechanism of transmission between badgers and cattle is uncertain (Godfray *et al.* 2013, Corner, Murphy & Gormley 2011), impeding the identification of promising management tools. Evidence that badgers and cattle seldom come into close contact (Böhm, Hutchings & White 2009, Mullen *et al.* 2013, Drewe *et al.* 2013, O'Mahony 2014, Woodroffe *et al.* 2016) has led to the suggestion that indirect transmission through the shared environment (as can occur between deer and cattle, Palmer, Waters & Whipple 2004) may be more important than direct transmission. If this inference were correct, it would help to inform both modelling and management of cattle TB.

There is no doubt that both badgers and cattle can shed *M. bovis* into the environment. Viable *M. bovis* has been recovered from the faeces, urine, and sputum of naturally-infected badgers (Clifton-Hadley, Wilesmith & Stuart 1993, Gallagher & Clifton-Hadley 2000), and from the faeces, and nasal and tracheal mucus, of naturally-infected cattle (Williams & Hoy 1927, de Kantor & Roswurm 1978, McIlroy, Neill & McCracken 1986). Such bacterial shedding can potentially contaminate pasture, cattle housing, drinking water, and soil (King *et al.* 2015, Barbier *et al.* 2016).

There is also evidence that *M. bovis* can persist in the environment shared by badgers and cattle. Field experiments have repeatedly demonstrated environmental persistence of *M. bovis* (Table S1); for example, Williams & Hoy (1930) infected guinea pigs with *M. bovis* extracted from the faeces of naturally-infected cattle up to four months after spreading it on cattle pasture. However, while many studies have emphasised the long-term detectability of *M. bovis* in the environment (e.g., Williams & Hoy 1930, Ghodbane *et al.* 2014), the numbers of bacteria detected per sample decline over the first few days of exposure to natural environmental conditions (Fine *et al.* 2011), and several studies have included samples in which *M. bovis* was detectable for only 2-4 days (Table S1). The risk of receiving an infectious dose of *M. bovis* is therefore likely to be greatest if contamination is encountered shortly (≤2 days) after it is deposited.

It is not clear how frequently badgers and cattle encounter fresh contamination during the hours or days when most bacteria are still viable. Cattle pasture is important badger habitat (Kruuk *et al.* 1979, Woodroffe et al. 2016), but badgers avoid cattle themselves (Benham & Broom 1989, Mullen et al. 2013, Woodroffe et al. 2016). Likewise, experimental studies have indicated that cattle avoid grazing on pasture contaminated with badger urine and faeces (Benham & Broom 1991, Hutchings & Harris 1997). If this mutual avoidance were to extend over several days, then the infectiousness of contamination might be waning by the time it was encountered. Hence, indirect contact might occur too infrequently to explain the interspecific transmission observed on TB-affected farmland (Donnelly & Nouvellet 2013, Woodroffe *et al.* 2006b).

We explored the possibilities for environmental transmission of *M. bovis* by investigating successive use of shared space by badgers and cattle. Using a large dataset of concurrent GPS-collar tracking of badgers and cattle (Woodroffe et al. 2016), we estimated how frequently each species used space occupied by the other ≤36h previously, and whether either species avoided space used recently by the other species.

Materials and Methods

Study sites

Data were collected at four sites in Cornwall, southwest Britain (C2, 50.6°N 4.4°W; C4, 50.6°N 4.8°W; F1, 50.2°N 5.6°W; F2, 50.1°N 5.3°W), between May 2013 and August 2015, as described in Woodroffe *et al.* (2016). Fieldwork was conducted with landholder permission, after ethical review by the Zoological Society of London, and under licence from Natural England and the UK Home Office. Each site comprised five farms, with ≥2 dairy and ≥2 beef herds per site, giving 20 farms in total (10 dairy, 10 beef; Table 1). Sites were >20km apart and all had *M. bovis* confirmed in both cattle and badgers (Woodroffe *et al.* 2017).

Data collection

We monitored both cattle and badger movements on the study farms (Table 1). Cattle were briefly restrained in a crush to fit GPS-collars (GPS-Plus, Vectronic Aerospace GmBH, Berlin). We conducted a herd tracking bout on each farm every few months, disinfecting collars between deployments on different herds. During each herd tracking bout, we aimed to monitor all cattle groups on the focal farm, (e.g., bullocks, milkers), with an average of 5.6 individual cattle (range 1-13) collared per tracking bout. We avoided collaring the same individual cattle on multiple tracking bouts. In total, 421 individual cattle were tracked, with a mean tracking bout length of 19.3 days (SD 23.1, range 1-213) before the collars were removed or dropped off.

We also used GPS-collars (Telemetry Solutions, Concord, CA, USA) to monitor badger movements. Badgers were cage-trapped, and chemically immobilised for collaring following de Leeuw *et al.* (2004). We aimed to maintain a collar on at least one badger per social group throughout the study; in total, 54 individual badgers were tracked (Table 1), with a mean collar deployment period of 110 nights (SD 74, range 4-296) before the collar battery expired, the collar was removed, or the badger died or disappeared. As for cattle, we avoided collaring the same individual repeatedly; as a result, 48% of the adult badgers captured during the study wore a GPS-collar at some point. Trapping was conducted to remove as many collars as possible at the end of the study.

Cattle and badger collars recorded GPS-locations at the same predetermined times, 20 mins apart (e.g., 2320h, 2340h), both outdoors and inside farm buildings (Woodroffe et al. 2016). Cattle collars recorded locations 24h per day, but badger collars attempted GPS-locations only between 1800h and 0600h UTC (Figure 1(a)), and only if an integral accelerometer indicated that the badger was active (Woodroffe et al. 2016). Following Woodroffe *et al.* (2016), we filtered GPS-collar data from both species to remove potentially inaccurate locations; details of this filtering are provided in the Supplementary Material. After filtering, GPS-collar locations were on average accurate to 4.7m (95% confidence interval (CI) 4.5-4.9m) for badgers, and 4.6m (95% CI 4.5-4.7m) for cattle.

Distances between badger and cattle locations

We identified individual collared badgers and cattle with the opportunity to interact, by determining which cattle were located within badger home ranges (measured as minimum convex polygons) during badger monitoring periods. For each sympatric badger-cattle pair we then calculated, for each 12h badger-tracking night (1800h-0600h), the minimum distance between the nights' badger locations, and cattle locations from the preceding 24h-period (1800h-1800h; Figure 1(b)). Minimum distances were thus calculated between collar locations recorded ≤36h apart. We likewise calculated, for each 24h of cattle-tracking (0600h-0600h), the minimum distance between the 24h-period's cattle locations, and badger locations from the preceding night (1800h-0600h; Figure 1(c)).

These minimum distances were calculated without regard to where badgers and cattle were located within their shared range; hence they did not distinguish locations at sites thought likely to be contaminated (e.g., badger latrines) from those elsewhere. Badgers' latrine use is highly seasonal, and so the probability of cattle encountering fresh contamination at a latrine site would likewise vary over time. Cattle responses to active latrines were therefore explored separately (Ham 2019).

The ≤36h time separation was chosen as the shortest interval which would not be influenced by diel variation in movement behaviour. While badgers are nocturnal, cattle are more active during the daytime (Figure S1), hence the risk of each species encountering contamination left by the other is likely to vary with the time of day. By taking the minimum distance between the two species during entire daily monitoring periods for cattle (24h) and badgers (12h), we avoided potential impacts of diel variation on contact probability measured over shorter time intervals. The resulting ≤36h time separation fell within the minimum two-day survival time recorded for *M. bovis* in outdoor experiments (Table S1).

Avoidance of recently-used space

We compared cattle and badger use of space ≤36h apart with published evidence describing simultaneous space use from the same dataset (Woodroffe et al. 2016). We previously reported 5,380 badger-cattle nights of simultaneous tracking, but only one occasion when a collared badger was found <10m from one of the collared cattle (Woodroffe et al. 2016). We used chi-squared tests to compare this published proportion of simultaneous locations <10m apart with the proportions of 36h periods in this study which had minimum separation distances of <10m.

We used compositional analysis to test the hypothesis that each species avoided space occupied by the other ≤36h previously (Aebischer, Robertson & Kenward 1993). Based on the <5m location accuracy of the GPS-collars, we classified each minimum separation distance as <5m, 5-10m, 10-20m, 20-30m, 30-40m, 40-50m, or >50m. For each animal tracked, the proportions of minimum separation distances in each of these distance categories summed to 1 across all categories; such an array of proportions is termed a composition (Aebischer, Robertson & Kenward 1993). Compositional analysis entails comparing observed and expected compositions, and is usually used to explore animals' selection of discrete habitat types (Aebischer, Robertson & Kenward 1993). We calculated the observed composition for each individual badger as the proportions of tracking nights in which the minimum distance to collared cattle fell into each of the distance categories. We likewise calculated the observed composition for each cattle herd as the proportions of 24h tracking periods in which the minimum distance to a collared badger fell into each of the distance categories (grouping cattle into herds as the shorter tracking periods for individual cattle led to imprecise estimates of the proportions of 24h periods in each minimum distance category). As cattle cannot move freely between fields, we calculated cattle compositions using only badger-cattle-24h-periods when collared cattle occupied fields visited the previous night by a collared badger. A secondary analysis (shown in Supplementary Material) explored badger compositions including only cattle locations >25m from farm buildings, since badgers in our study areas seldom entered buildings (Woodroffe et al. 2017), so housed cattle may not have been available for contact.

We generated expected compositions by taking the same datasets of GPS-collar locations and permuting the monitoring periods. Thus, for each night of badger tracking, the minimum distance to collared cattle was calculated not for the preceding 24h of cattle tracking, but for another, randomly selected, 24h-period (Figure 1(b)). Likewise for each 24h of cattle tracking, the minimum distance to a collared badger was calculated for another, randomly selected, night of badger tracking (Figure 1(c)).

Permutations were repeated 20 times, generating 20 expected compositions for each badger, and for each cattle herd. We then used *Compos* (Smith 2005) to compare, separately, the observed compositions of minimum separation distances across all GPS-collared badgers, and all cattle herds, with each of their respective 20 expected compositions. We report the average *p*-value (with 95% CI) across each set of 20 compositional analyses.

Frequency of successive space use

To explain our methods with the greatest clarity, we here term successive space use by badgers and cattle as a "contact" event. Specifically, if a collared cow was located <5m from a location occupied by a badger ≤36h previously, the cow was considered (for the purposes of our analysis) to have <u>contacted</u> the badger, and the badger to have <u>been contacted by</u> the cow.

This <5m minimum separation distance was determined by the accuracy of the GPS-collars, and for three reasons does not provide a perfect representation of the true contact rate. First, two locations recorded <5m and ≤36h apart need not entail the two animals occupying precisely the same location. Second, each minimum separation distance may have been under-estimated because locations were only recorded every 20 minutes. Third, not all such contact events would be potentially infectious, as neither badgers nor cattle are likely to contaminate every location they occupy. Nevertheless, estimating the frequency of such "contacts" has heuristic value in exploring successive space use. Note that, by this definition, each badger-cattle pair could have only one contact event per 24h period.

We estimated the probability of one of the collared cattle contacting a collared badger (F; Table 2) as the proportion of badger-cattle-24h-periods when a contact was recorded. Using this estimated probability, we sought to estimate the frequency of contact between any member of a cattle herd, and any member of a badger social group, irrespective of whether the individuals were collared. Such scaling-up is sensitive to assumptions about whether individual cattle contact badgers independently of other cattle, and whether individual badgers are contacted by cattle independently of other badgers. We used our GPS-collar data to test these assumptions, as well as to estimate the frequency with which contact would occur under each set of assumptions.

At one extreme, if the badgers using a farm all used exactly the same locations each night (not necessarily simultaneously), then any of the collared cattle which contacted one collared badger would also contact all the other badgers using that farm. In this extreme scenario, individual collared cattle would contact any badger (collared or uncollared) with the same probability as they contacted individual collared badgers (F). At the other extreme, if there were AB badgers using the farm (A social groups, each comprising B badgers; Table 2), which each moved independently of one another, then individual collared cattle would contact any badger (collared or uncollared) with frequency $1\text{-}(1\text{-}F)^{AB}$ (Table 2).

Taking the same logic a step further, if cattle within a herd all used exactly the same locations as one another, then any of the cattle on a farm (collared or uncollared) would contact any badger (collared or uncollared) with the same probability as do collared cattle (i.e., either F or $1\text{-}(1\text{-}F)^{AB}$, as described above). However, if there were C cattle within a herd which moved independently of one another, then the probability of any cattle within the herd (collared or uncollared) contacting any badger (collared or uncollared) would be $1\text{-}(1\text{-}F)^{C}$ if badgers used all the same locations, and $1\text{-}[(1\text{-}F)^{AB}]^{C}$ if badgers moved independently (Table 2). These formulae allowed us to estimate the probability of any of the cattle in a herd contacting any badger using the farm, separately for four different contact scenarios representing different assumptions about the independent movement of conspecific individuals (Table 2).

To estimate the probability of each of these contact scenarios occurring, we first explored whether individual badgers using a farm were contacted by cattle independently of other badgers. Individual badgers might be indirectly contacted together even if they did not move together, for example if they consistently visited the same badger latrines or water troughs. We counted how many badger-cattle-24h-periods entailed simultaneous tracking of multiple collared badgers using the same farm and, among these periods, how many resulted in contacts by collared cattle. We then used maximum likelihood to compare these observed frequencies of multiple contact with three possible expected frequencies. Each of the expected frequencies was calculated in a Microsoft Excel spreadsheet, using maximum likelihood to estimate the model and parameter values with the best fit to the data (Spreadsheet S1). We calculated the first expected frequency by assuming that collared badgers were contacted independently of one another. We calculated the second expected frequency by fitting a mixture model, which assumed that collared badgers were contacted by collared cattle together on a proportion (G, to be estimated) of occasions and were otherwise contacted independently. We calculated a third expected frequency using another mixture model, which assumed that collared badgers were contacted by collared cattle independently on a proportion (to be estimated) of occasions and were otherwise avoided after the initial contact (i.e., if one was contacted, the others were not). These mixture models avoided problems associated with expected values of zero within simpler models assuming that collared badgers were encountered all together, or were avoided after the first encounter (Spreadsheet S1).

We likewise explored whether collared cattle contacted badgers independently of other cattle. We counted how many badger-cattle-24h-periods entailed simultaneous tracking of collared cattle in the same herd and, among these, how many times one or more collared cattle contacted badgers. We then used the frequency of contact when only one of the cattle was collared (i.e. the first row in Table S2) to calculate expected contact frequencies when multiple cattle were collared, assuming collared animals made contacts independently of other members of their own herd. We then compared observed and expected contact frequencies in Excel using maximum likelihood, to estimate the proportion of 24h periods (I) when individual cattle contacted badgers together with other herd members.

These calculations provided, for each of four scenarios, an estimate of the probability of any cattle within a herd contacting any badger using the farm, and an estimate of the probability of that scenario occurring (Table 2; Spreadsheet S2). We then multiplied the probability of each scenario occurring by the contact probability associated with that scenario (Table 2). The sum of the resulting four numbers provided an estimate of the overall probability of contact (Table 2). We likewise estimated the number of contacts per unit time for each scenario, and for all scenarios combined (Table 2; Spreadsheet S2). We conducted sensitivity analyses by replacing the estimates of F, G and I with their upper and lower 95% confidence limits.

We repeated these calculations to estimate the frequency of any badger in a social group contacting any of the cattle using their home range (Table 3; Spreadsheet 2). However, we could not test whether collared badgers contacted cattle independently of other badgers on the same farm, as there were no nights when more than one collared badger on the same farm contacted the same individual among the collared cattle. We therefore explored two extreme scenarios assuming that badgers either contacted cattle independently of other badgers, or that all badgers contacted the same cattle (Table 3; Spreadsheet S2).

Results

Across our four study sites, we monitored 421 GPS-collared cattle over 8,551 cattle-24h-periods, and 54 GPS-collared badgers over 7,176 badger-nights (Table 1). Thirty-eight of the 54 GPS-collared badgers had the opportunity to interact with 278 of the 421 GPS-collared cattle in 468 unique badger-cattle pairs. Within these badger-cattle pairs, there were 5,877 badger-cattle-24h-periods when cattle were tracked after a night of badger tracking, and 5,307 cattle-badger-nights when badgers were tracked after 24h of cattle tracking (Table 1).

Both species were recorded <5m from locations occupied by the other species ≤36h previously. Such contacts between GPS-collared individuals were recorded on 67 of 5,877 badger-cattle-24h-periods when cattle were tracked after badgers (Table 1); the shortest estimated separation distance was 0.5m. Likewise, contacts were recorded on 62 of 5,307 cattle-badger-nights when badgers were tracked after cattle (Table 1); the shortest estimated separation distance was 0.1m.

Avoidance of recently occupied space

Collared badgers and cattle used space occupied by the other species ≤36h previously far more often than they used space simultaneously. In a previous paper (Woodroffe et al. 2016) we found no simultaneous locations <5m apart, and only one occasion when collared badgers and cattle were located <10m apart in 5,380 badger-cattle-nights of simultaneous tracking. This frequency (1/5,380 badger-cattle-nights) was significantly lower than both the frequency of cattle being recorded <10m from locations occupied by badgers in the previous 36h (163/5,877 badger-cattle-24h-periods; χ^2=148.5, df=1, p<0.001), and the frequency of badgers being recorded <10m from locations occupied by cattle in the previous 36h (162/5,307 cattle-badger-nights; χ^2=163.7, df=1, p<0.001).

Compositional analyses revealed no evidence that either cattle or badgers avoided space occupied by the other species ≤36h previously. The distribution of badger and cattle locations across separation distance categories did not differ significantly between paired locations ≤36h apart and paired locations separated by randomly-selected time periods (Figure 2; badger locations relative to cattle, mean p value=0.343, 95% CI 0.259-0.426; cattle locations relative to badgers in the same field, mean p value=0.476, 95% CI 0.376-0.576). Secondary analyses showed similar results when cattle data were restricted to locations >25m outside farm buildings (see Supplementary Material). The relatively high proportion of separation distances of 10-30m shown in Figure 2 occurs because these were minimum distances; far fewer short distances were observed in the frequency distribution of mean separation distances (Figure S2).

Frequency of successive space use

We estimated that, depending on assumptions about whether individual animals (of either species) moved independently or together, the average herd would contact one or more badgers between 0.0114 and 11.5 times per 24h period (Table 2; Spreadsheet S2).

Although this range of contact frequencies is very wide, our analyses suggested that some scenarios were much more likely than others. When each of the collared cattle was tracked simultaneously with multiple collared badgers (Table S3), the best fit model suggested that badgers were contacted independently on 61.9% (95% CI 45.9-78.6%) of occasions and together on 38.1% (95% CI 21.4-54.1%) of occasions (Table S4; Table 2; Spreadsheet S1). Likewise, when multiple collared cattle were tracked simultaneously with the same collared badger (Table S5), the observed proportion of 24h periods when >1 cattle contacted the same badger (8/1395; Table S2) was significantly higher than the proportion that would be expected if cattle moved independently (Table S2, 0.6/1395, χ^2=27.6, p<0.001). This finding indicates that collared cattle did not contact collared badgers independently of other cattle in their herds. Among 49 24h-periods when >1 collared cattle could have contacted a collared badger, and at least one of them did so, there was only one 24h-period when all of the collared cattle contacted the badger (Table S2). Hence, we conservatively estimated the probability of all the cattle in a herd contacting the same badger, given that one of them made such contact, as 1/49 (0.0204; exact binomial 95% CI 0.0005-0.1085) and the probability of this event not occurring as 48/49 (0.9796; exact binomial 95% CI 0.8915-0.9995; Table 2). These values almost certainly over-estimate the probability of all the cattle in a herd contacting the same badger, since the one occasion when all collared cattle contacted the same badger occurred when only two cattle were collared (Table S2), whereas the average herd comprised 176 cattle (Table 1).

Multiplying the probability of each of the four scenarios occurring by the corresponding probability of contact, and summing the four products, gave an overall contact probability of 0.9308 per 24h (Table 2). We estimated that, on average, a cattle herd would contact badgers 7.7 times per 24h period (Table 2; Spreadsheet S2). Sensitivity analyses generated estimates between 6.0 and 9.8 contact events per 24h period (Table S6).

A similar set of calculations (Table 3; Spreadsheet S2) applied to badgers contacting cattle. When collared badgers were tracked simultaneously with multiple collared cattle, the observed pattern of contacts (Table S7) was best described by a mixture model in which badgers contacted each of the collared cattle independently on 25.2% of nights (95% CI 19.2-32.2%) and together on 74.8% of nights (95% CI 67.8-80.8%; Table S4; Spreadsheet S1). We could not test whether individual badgers were independent of one another in their probabilities of contacting cattle, because there were no incidents when more than one collared badger from the same social group contacted the same individual among the collared cattle (Table S8). The expected number of cattle-nights with such multiple contacts was very low, however (0.03; Table S9). As we could not test whether collared badgers contacted cattle independently of other badgers in the same social group, we explored two extreme assumptions, with badgers either contacting cattle completely independently of other badgers ($i=1$, $j=0$, Table 3; Spreadsheet S2), or all contacting the same cattle ($i=0$, $j=1$, Table 3; Spreadsheet S2). Combining the four scenarios gave an overall contact probability between 0.256 and 0.282, depending on assumptions (Table 3). These calculations indicated that, on average, a badger social group would contact cattle between 0.99 and 3.5 times per night, depending on assumptions (Table 3; Spreadsheet S2) . Sensitivity analyses generated estimates between 0.76 and 4.5 contact events per night (Table S10).

Discussion

The movement patterns we observed among cattle and badgers suggest that both species could potentially encounter environmental contamination left by the other, well within the survival period of *M. bovis*. Even though *M. bovis* may only survive in the environment for <72h under some circumstances (Fine et al. 2011), we found that both badgers and cattle were frequently located in space occupied by the other species during the previous 36h. By our definition of contact, we estimated that cattle herds contacted badgers at least 6.0 times per 24h on average, and badger social groups contacted cattle at least 0.76 times per night, with neither species avoiding contact with the other. Had we used a longer time window to define a contact, as might have been justified by the capacity for prolonged survival of *M. bovis* in the environment (Table S1). contact rates would have been even higher.

These estimates of indirect contact rates should be interpreted with caution, as they are extrapolated from small numbers of observed contacts. Nevertheless, they clearly contrast with our previously-published estimates of the frequency of direct contact (Woodroffe et al. 2016). Close proximity (<10m) between the locations of GPS-collared animals ≤36h apart occurred two orders of magnitude more frequently than close proximity between simultaneous locations of the same animals. Hence, if there was any (undetected) avoidance of recently-occupied space, it was much weaker than avoidance of animals themselves.

Importantly, for such contacts to be infectious, *M. bovis* would have to remain in the environment after the host animal has moved elsewhere, probably in faeces, urine, sputum, or mucus. On most farms, most badgers and most cattle are not infected with *M. bovis* (Woodroffe *et al.* 2005), and would leave no such contamination. Moreover, where infected individuals are present, the risk of infectious contact is likely to vary between hosts species and between forms of contamination. As cattle do not select specific areas to defecate or urinate (White *et al.* 2001), they could potentially contaminate any location where they have been present. Likewise, their sputum or nasal mucus could potentially contaminate anywhere they have fed or drunk (Palmer, Waters & Whipple 2004). Hence, cattle contamination might occur almost anywhere on farmland, and the distribution of cattle GPS-locations may approximate to the spatial distribution of interspecific transmission risk to badgers. Similarly, badger sputum and mucus could contaminate any site where badgers have fed or drunk, which may occur almost anywhere in the farm environment (Kruuk et al. 1979, Garnett, Delahay & Roper 2002), so that the distribution of badger GPS-locations would reflect the spatial distribution of transmission risk from badgers to cattle via these secretions. However, badgers tend to concentrate faeces and urine at latrines (White, Brown & Harris 1993, Kruuk 1978, Roper *et al.* 1993), so the spatial distribution of badger-to-cattle transmission risk from these sources would not reflect the distribution of badger GPS-collar locations. Badger latrines have been recognised as sites of potential *M. bovis* transmission to cattle (Drewe et al. 2013), but their importance relative to the wider farm environment has not been quantified. For this reason, any close proximity between badger and cattle locations may entail some potential risk of *M. bovis* transmission, wherever it occurs.

The strong possibility that *M. bovis* transmission between badgers and cattle can occur through the environment could help to explain the apparent sensitivity of cattle TB incidence to changes in badger territorial behaviour (Woodroffe *et al.* 2006a, Donnelly *et al.* 2006). At low population densities, badgers appear less likely to use latrines, with isolated urinations and defecations found more frequently on pasture where they are more accessible to cattle (Hutchings, Service & Harris 2002). Hence, while culling reduces badger density, it may not proportionally reduce cattle exposure to badgers. This observation may help to explain why changes in cattle TB incidence associated with badger culling are not proportional to reductions in badger density (Woodroffe *et al.* 2008).

While our study focused on *M. bovis* transmission between species, if both species contaminate the environment and can become infected when they encounter such contamination, it is possible that transmission within species might also occur through an environmental route. We did not estimate within-species contact rates, but our evidence of non-independent movement by both badgers and cattle (Table S4) suggests that such contact rates would be high.

If environmental transmission (whether from other cattle or from badgers) accounts for a proportion of new TB incidents in cattle, then environmental conditions which facilitate *M. bovis* survival could play an important role in TB dynamics (King, Lovell & Harris 1999). Existing dynamical models of TB in badgers and cattle either do not account for bacterial survival in the environment (Anderson & Trewhella 1985, Smith *et al.* 2001, Hardstaff *et al.* 2012), or do not distinguish it from persistence within the badger population (Brooks-Pollock, Roberts & Keeling 2014). Given substantial variation in when, where and how many bacteria are shed into the environment, how many survive and for how long, whether they remain in a form that can be aerosolised, and whether and how they are encountered by a susceptible host, environmental transmission is likely to be highly stochastic.

Our findings have important implications for TB control. Cattle excretory behaviour means that cattle contamination is unlikely to be localised (White et al. 2001); indeed practices such as slurry spreading may distribute it beyond the movements of cattle themselves (McCallan, McNair & Skuce 2014). Excluding badgers from space contaminated by cattle is thus unlikely to be practical, and so cattle-to-badger transmission may be controllable only through badger vaccination or management to reduce *M. bovis* prevalence in cattle. In contrast, badger contamination might potentially be concentrated at specific sites, such as latrines (where most defecation and urination occurs, Kruuk 1978, White, Brown & Harris 1993) or water troughs (where *M. bovis* survival may be especially high, Fine et al. 2011). Identifying such transmission sites is a priority for TB management. For example, if badger latrines appear to be important sites of transmission, then fencing cattle away from latrines might reduce their TB risks, yet such management would be ineffective if most transmission occurred through contaminated drinking water. Methods are available to detect *M. bovis* in the environment (King et al. 2015), and could be usefully combined with analyses of cattle and badger movement behaviour to identify sites where interspecific transmission is most likely.

Author Contributions

RW designed and coordinated the study, participated in data collection and analysis, and drafted the manuscript. CAD oversaw statistical analyses. CH, KM, KC and NS contributed to study design and data collection. SC helped design and conduct the statistical analyses. All authors approved submission.

Acknowledgements

We thank landholders for allowing fieldwork access, and Defra for funding. CAD thanks the Medical Research Council for Centre Funding. We declare no competing interests.

Data accessibility

Data will be deposited on Dryad on acceptance. All tracking data are lodged on Movebank (www.movebank.org; Movebank Project 158275131).

References

Aebischer, N. J., P. A. Robertson & R. E. Kenward (1993) Compositional analysis of habitat use from animal radio-tracking data. *Ecology,* **74,** 1313-1325.

Anderson, R. M. & W. Trewhella (1985) Population dynamics of the badger (*Meles meles*) and the epidemiology of bovine tuberculosis (*Mycobacterium bovis*). *Philosophical Transactions of the Royal Society of London, Series B,* **310,** 327-381.

Barbier, E., B. Chantemesse, M. Rochelet, L. Fayolle, L. Bollache, M. L. Boschiroli & A. Hartmann (2016) Rapid dissemination of *Mycobacterium bovis* from cattle dung to soil by the earthworm *Lumbricus terrestris*. *Veterinary Microbiology,* **186,** 1-7.

Benham, P. F. J. & D. M. Broom (1989) Interactions between cattle and badgers at pasture with reference to bovine tuberculosis transmission. *British Veterinary Journal,* **145,** 226-241.

Benham, P. F. J. & D. M. Broom (1991) Responses of dairy cows to badger urine and faeces on pasture with reference to bovine tuberculosis transmission. *British Veterinary Journal,* **147,** 517-531.

Böhm, M., M. R. Hutchings & P. C. L. White (2009) Contact networks in a wildlife-livestock host community: identifying high-risk individuals in the transmission of bovine TB among badgers and cattle. *PLOS One,* **4,** e5016.

Breban, R. (2013) Role of environmental persistence in pathogen transmission: a mathematical modeling approach. *Journal of Mathematical Biology,* **66,** 535-546.

Brooks-Pollock, E., G. O. Roberts & M. J. Keeling (2014) A dynamic model of bovine tuberculosis spread and control in Great Britain. *Nature,* **511,** 228-231.

Brooks-Pollock, E. & J. L. N. Wood (2015) Eliminating bovine tuberculosis in cattle and badgers: insight from a dynamic model. *Proceedings of the Royal Society B-Biological Sciences,* **282,** 20150374.

Clifton-Hadley, R. S., J. W. Wilesmith & F. A. Stuart (1993) *Mycobacterium bovis* in the European badger (*Meles meles*): Epidemiological findings in tuberculous badgers from a naturally infected population. *Epidemiology & Infection,* **111,** 9-19.

Corner, L. A. L., D. Murphy & E. Gormley (2011) *Mycobacterium bovis* infection in the Eurasian Badger (*Meles meles*): the disease, pathogenesis, epidemiology and control. *Journal of Comparative Pathology,* **144,** 1-24.

Crispell, J., C. H. Benton, D. Balaz, N. De Maio, A. Ahkmetova, A. Allen, R. Biek, E. L. Presho, J. Dale, G. Hewinson, S. J. Lycett, J. Nunez-Garcia, R. A. Skuce, H. Trewby, D. J. Wilson, R. N. Zadoks, R. J. Delahay & R. R. Kao (2019) Combining genomics and epidemiology to analyse bi-directional transmission of Mycobacterium bovis in a multi-host system. *Elife,* **8,** 36.

de Kantor, I. N. & J. D. Roswurm (1978) Mycobacteria isolated from nasal secretions on tuberculin test reactor cattle. *American Journal of Veterinary Research,* **39,** 1233-1234.

de Leeuw, A. N. S., G. J. Forrester, P. D. Spyvee, M. G. I. Brash & R. J. Delahay (2004) Experimental comparison of ketamine with a combination of ketamine, butorphanol and medetomidine for general anaesthesia of the Eurasian badger (*Meles meles* L.). *Veterinary Journal,* **167,** 186-193.

Defra (2014) *The Strategy for achieving Officially Bovine Tuberculosis Free status for England*. https://www.gov.uk/government/uploads/system/uploads/attachment_data/file/300447/pb14088-bovine-tb-strategy-140328.pdf.

Donnelly, C. A. & P. Nouvellet (2013) The contribution of badgers to confirmed tuberculosis in cattle in high incidence areas in England. *PLoS Currents Outbreaks*.

Donnelly, C. A., R. Woodroffe, D. R. Cox, F. J. Bourne, C. L. Cheeseman, G. Wei, G. Gettinby, P. Gilks, H. Jenkins, W. T. Johnston, A. M. Le Fevre, J. P. McInerney & W. I. Morrison (2006) Positive and negative effects of widespread badger culling on cattle tuberculosis. *Nature,* **439,** 843-846.

Drewe, J. A., H. M. O'Connor, N. Weber, R. A. McDonald & R. J. Delahay (2013) Patterns of direct and indirect contact between cattle and badgers naturally infected with tuberculosis. *Epidemiology and Infection,* **141,** 1467-1475.

Fine, A. E., C. A. Bolin, J. C. Gardiner & J. B. Kaneene (2011) A study of the persistence of *Mycobacterium bovis* in the environment under natural weather conditions in Michigan, USA. *Veterinary medicine international,* **2011,** 765430-765430.

Gallagher, J. & R. S. Clifton-Hadley (2000) Tuberculosis in badgers: a review of the disease and its significance for other animals. *Research in Veterinary Science,* **69,** 203-217.

Garnett, B. T., R. J. Delahay & T. J. Roper (2002) Use of cattle farm resources by badgers (*Meles meles*) and risk of bovine tuberculosis (*Mycobacterium bovis*) transmission to cattle. *Proceedings of the Royal Society of London Series B-Biological Sciences,* **269,** 1487-1491.

Ghodbane, R., F. M. Medie, H. Lepidi, C. Nappez & M. Drancourt (2014) Long-term survival of tuberculosis complex mycobacteria in soil. *Microbiology-Sgm,* **160,** 496-501.

Godfray, H. C. J., C. A. Donnelly, R. R. Kao, D. W. Macdonald, R. A. McDonald, G. Petrokofsky, J. L. N. Wood, R. Woodroffe, D. B. Young & A. R. McLean (2013) A restatement of the natural science evidence base relevant to the control of bovine tuberculosis in Great Britain. *Proceedings of the Royal Society B-Biological Sciences,* **280,** 20131634.

Ham, C. (2019) *The ecology of pathogen transmission between wildlife and livestock*. PhD thesis, Imperial College London.

Hardstaff, J. L., M. T. Bulling, G. Marion, M. R. Hutchings & P. C. L. White (2012) Impact of external sources of infection on the dynamics of bovine tuberculosis in modelled badger populations. *BMC veterinary research,* **8,** 92-92.

Hutchings, M. R. & S. Harris (1997) Effects of farm management practices on cattle grazing behaviour and the potential for transmission of bovine tuberculosis from badgers to cattle. *The Veterinary Journal,* **153,** 149-162.

Hutchings, M. R., K. M. Service & S. Harris (2002) Is population density correlated with faecal and urine scent marking in European badgers (*Meles meles*) in the UK? *Mammalian Biology,* **67,** 286-293.

Joh, R. I., H. Wang, H. Weiss & J. S. Weitz (2009) Dynamics of indirectly transmitted infectious diseases with immunological threshold. *Bulletin of Mathematical Biology,* **71,** 845-862.

King, E. J., D. J. Lovell & S. Harris, (1999) Effect of climate on the survival of *Mycobacterium bovis* and its transmission to cattle herds in south-west Britain. In: *Advances in vertebrate pest management*: 147-161. D. P. Cowan & C. J. Feare (Eds.). Filander Verlag, Fürth.

King, H. C., A. Murphy, P. James, E. Travis, D. Porter, Y. J. Hung, J. Sawyer, J. Cork, R. J. Delahay, W. Gaze, O. Courtenay & E. M. Wellington (2015) The variability and seasonality of the environmental reservoir of *Mycobacterium bovis* shed by wild European badgers. *Scientific Reports,* **5,** 7.

Kruuk, H. (1978) Spatial organization and territorial behaviour of the European badger *Meles meles*. *Journal of Zoology,* **184,** 1-19.

Kruuk, H., T. Parish, C. A. J. Brown & J. Carera (1979) The use of pasture by the European badger (*Meles meles*). *Journal of Applied Ecology,* **16,** 453-459.

McCallan, L., J. McNair & R. Skuce (2014) *A review of the potential role of cattle slurry in the spread of bovine tuberculosis*. Belfast: http://www.dardni.gov.uk/tb-slurry-lit-review.pdf.

McIlroy, S. G., S. D. Neill & R. M. McCracken (1986) Pulmonary lesions and *Mycobacterium bovis* excretion from the respiratory tract of tuberculin reacting cattle. *Veterinary Record,* **118,** 718-721.

Mullen, E. M., T. MacWhite, P. K. Maher, D. J. Kelly, N. M. Marples & M. Good (2013) Foraging Eurasian badgers *Meles meles* and the presence of cattle in pastures. Do badgers avoid cattle? *Applied Animal Behaviour Science,* **144,** 130-137.

O'Mahony, D. T. (2014) *Badger-cattle interactions in the rural environment: implications for bovine tuberculosis transmission*. Belfast: Agri-food and Biosciences Institute: http://www.dardni.gov.uk/badger-cattle-proximity-report.pdf.

Palmer, M. V., W. R. Waters & D. L. Whipple (2004) Investigation of the transmission of *Mycobacterium bovis* from deer to cattle through indirect contact. *American Journal of Veterinary Research,* **65,** 1483-1489.

Pritchard, D. G. (1988) A century of bovine tuberculosis 1888-1988 – conquest and controversy. *Journal of Comparative Pathology,* **99,** 357-399.

Rohani, P., R. Breban, D. E. Stallknecht & J. M. Drake (2009) Environmental transmission of low pathogenicity avian influenza viruses and its implications for pathogen invasion. *Proceedings of the National Academy of Sciences of the United States of America,* **106,** 10365-10369.

Roper, T. J., L. Conradt, J. Butler, S. F. Christian, J. Ostler & T. K. Schmid (1993) Territorial marking with faeces in badgers (*Meles meles*): a comparison of boundary and hinterland latrine use. *Behaviour,* **127,** 289-307.

Smith, G. C., C. L. Cheeseman, D. Wilkinson & R. S. Clifton-Hadley (2001) A model of bovine tuberculosis in the badger *Meles meles*: the inclusion of cattle and the use of a live test. *Journal of Applied Ecology,* **38,** 520-535.

Smith, P. G. (2005) *Compos Analysis, version 6.2 plus*. Abergavenny, UK: Smith Ecology Ltd.

White, P. C. L., J. A. Brown & S. Harris (1993) Badgers (*Meles meles*), cattle and bovine tuberculosis: a hypothesis to explain the influence of habitat on the risk of disease transmission in southwest England. *Proceedings of the Royal Society of London Series B-Biological Sciences,* **253,** 277-284.

White, S. L., R. E. Sheffield, S. P. Washburn, L. D. King & J. T. Green (2001) Spatial and time distribution of dairy cattle excreta in an intensive pasture system. *Journal of Environmental Quality,* **30,** 2180-2187.

Williams, R. S. & W. A. Hoy (1927) Tubercle bacilli in the faeces of apparently healthy cows. *Journal of Hygiene,* **27,** 37-39.

Williams, R. S. & W. A. Hoy (1930) The viability of *B. tuberculosis* (*bovinus*) on pasture land, in stored faeces and in liquid manure. *Journal of Hygiene,* **30,** 413-419.

Woodroffe, R., C. A. Donnelly, D. R. Cox, F. J. Bourne, C. L. Cheeseman, R. J. Delahay, G. Gettinby, J. P. McInerney & W. I. Morrison (2006a) Effects of culling on badger (*Meles meles*) spatial organization: implications for the control of bovine tuberculosis. *Journal of Applied Ecology,* **43,** 1-10.

Woodroffe, R., C. A. Donnelly, C. Ham, S. Y. B. Jackson, K. Moyes, K. Chapman, N. G. Stratton & S. J. Cartwright (2016) Badgers prefer cattle pasture but avoid cattle: implications for bovine tuberculosis control. *Ecology Letters,* **19,** 1201-1208.

Woodroffe, R., C. A. Donnelly, C. Ham, S. Y. B. Jackson, K. Moyes, K. Chapman, N. G. Stratton & S. J. Cartwright (2017) Use of farm buildings by wild badgers: implications for the transmission of bovine tuberculosis. *European Journal of Wildlife Research,* **63,** 6.

Woodroffe, R., C. A. Donnelly, H. E. Jenkins, W. T. Johnston, D. R. Cox, F. J. Bourne, C. L. Cheeseman, R. J. Delahay, R. S. Clifton-Hadley, G. Gettinby, P. Gilks, R. G. Hewinson, J. P. McInerney & W. I. Morrison (2006b) Culling and cattle controls influence tuberculosis risk for badgers. *Proceedings of the National Academy of Sciences of the United States of America,* **103,** 14713-14717.

Woodroffe, R., C. A. Donnelly, W. T. Johnston, F. J. Bourne, C. L. Cheeseman, R. S. Clifton-Hadley, D. R. Cox, G. Gettinby, R. G. Hewinson, A. M. Le Fevre, J. P. McInerney & W. I. Morrison (2005) Spatial association of *Mycobacterium bovis* infection in cattle and badgers. *Journal of Applied Ecology,* **42,** 852-862.

Woodroffe, R., P. Gilks, W. T. Johnston, A. M. Le Fevre, D. R. Cox, C. A. Donnelly, F. J. Bourne, C. L. Cheeseman, G. Gettinby, J. P. McInerney & W. I. Morrison (2008) Effects of culling on badger abundance: implications for tuberculosis control. *Journal of Zoology,* **274,** 28-37.

Table 1 Summary of monitoring across four sites.

	Site				
	C2	C4	F1	F2	Overall
Years monitored	2013-5	2014-5	2013-5	2013-5	
Cattle monitoring					
Herds monitored (beef, dairy)	5 (3,2)	5 (2,3)	5 (3,2)	5 (2,3)	**20**
Cattle GPS-collared	171	21	150	79	**421**
Mean herd size	201.5	115.9	164.5	223.5	**176.3**
Mean farm size (sq km)*	1.18	0.78	1.07	1.55	**1.15**
24h-periods of GPS-collar monitoring	2,973	410	3,296	1,872	**8,551**
Badger monitoring					
Social groups monitored	6	5	7	10	**28**
Badgers GPS-collared	12	6	16	20	**54**
Mean social group size (minimum number alive)	2.3	2.4	5.6	3.4	**3.54**
Mean home range size (sq km)*	0.55	0.28	0.55	0.43	**0.45**
Nights of GPS-collar monitoring	1,397	511	2,585	2,683	**7,176**
Successive monitoring of GPS-collared badgers and cattle with overlapping ranges					
Badger-cattle-24h-periods (cattle tracked after badgers)	1,852	238	2,690	1,097	**5,877**
Badger-cattle-24h-periods when cattle <5m from badger location	5	10	49	3	**67**
Cattle-badger-nights (badgers tracked after cattle)	1,704	208	2,337	1,058	**5,307**
Cattle-badger-nights when badgers <5m from cattle location	9	6	42	5	**62**
Range overlap					
Badger social group territories per farm (mean, range)	2.8 (2-4)	1.3 (1-2)	3.2 (2-5)	3.8 (2-6)	**2.8**
Cattle herds per badger social group	2.6 (1-5)	1.3 (1-2)	3.0 (2-5)	1.7 (1-3)	**2.1**

*estimates from Ham (2019).

Table 2 – Estimating the frequency with which the average cattle herd contacted badgers, with a "contact event" defined as cattle being located <5m from a location occupied by a badger within the previous 36h. Full calculations are provided in Spreadsheet S2.

Background data	
Badger social group territories overlapping with each of the collared cattle (A)	1.62
Badger social group size (B)	3.54
Badgers which could potentially contact each of the collared cattle (A x B)	5.73
Cattle herd size (C)	176
Probability of collared cattle contacting collared badgers in a 24h-period	
Badger-cattle-24h tracking periods (D)	5,877
Badger-cattle -24h-periods with minimum separation distances <5m ("contact events", E)	67
Contact frequency ($E/D=F$)	0.0114
Proportion of 24h periods when badgers are contacted all together (G; Table S4)	0.381
Proportion of 24h periods when badgers are contacted independently (H; Table S4)	0.619
Proportion of 24h periods when herd members contact badgers together (I; see text)	0.0204
Proportion of 24h periods when herd members contact badgers independently (J; see text)	0.9796
Probability of collared cattle contacting any (collared & uncollared) badgers	
If badgers are all contacted together (F)	0.0114
If each badger is contacted independently of other badgers ($1-(1-F)^{AB}$)	0.0636
Probability of any cattle in a herd (collared & uncollared) contacting any badger	
<u>Scenario 1C: Cattle all move together and badgers are all contacted together</u>	
Probability that all the cattle in a herd contact badgers in this scenario (F)	0.0114
Average number of herd contact events per 24h period in this scenario (F)	0.0114
Proportion of 24h periods when this scenario occurs (I x G=$K1$)	0.0078
Proportion of all 24h periods with at least one contact of this type (I x G x F = K)	0.0001
<u>Scenario 2C: Cattle move independently and badgers are all contacted together</u>	
Probability that at least one of the cattle in a herd contact badgers in this scenario ($1-(1-F)^{C}$)	0.8671
Average number of herd contact events per 24h period in this scenario (C x F)	2.0065
Proportion of 24h periods when this scenario occurs (J x G=$L1$)	0.3732
Proportion of all 24h periods with at least one contact of this type (J x G x $[1-(1-F)^{C}] = L$)	0.3236
<u>Scenario 3C: Cattle all move together and badgers are contacted independently</u>	
Probability that all the cattle in a herd contact badgers in this scenario ($1-(1-F)^{AB}$)	0.0636
Average number of herd contact events per 24h period in this scenario (A x B x F)	0.0654
Proportion of 24h periods when this scenario occurs (I x H = $M1$)	0.0126
Proportion of all 24h periods with at least one contact of this type (I x H x $[1-(1-F)^{AB}] = M$)	0.0008
<u>Scenario 4C: Cattle move independently and badgers are contacted independently</u>	
Probability that any cattle in a herd contact badgers in this scenario ($1-[(1-F)^{AB}]^{C}$)	1.000
Average number of herd contact events per 24h period in this scenario (A x B x C x F)	11.507
Proportion of 24h periods when this scenario occurs (J x H = $N1$)	0.6064
Proportion of all 24h periods with at least one contact of this type (J x H x $[1-[(1-F)^{AB}]^{C}] = N$)	0.6064
Proportion of 24h periods with at least one contact event (K+L+M+N)	0.9308
Average number of contact events per 24h period ($[F$ x $K1]+[C$ x F x $L1]+[A$ x B x F x $M1]+[A$ x B x C x F x $N1]$)	7.7271

Table 3 – Estimating the frequency with which the average badger social group contacted cattle, with a “contact event” defined as a badger being located <5m from a location occupied by cattle within the previous 36h. Full calculations are provided in Spreadsheet S2.

Background data	
Cattle herds overlapping with each of the collared badgers (*a*)	1.89
Cattle herd size (*b*)	176
Cattle which could potentially contact each of the collared badgers (*a* x *b*)	332.6
Badger social group size (*c*)	3.54
Probability of collared badgers contacting collared cattle each night	
Cattle-badger-nights of tracking (*d*)	5,307
Cattle-badger-nights with minimum separation distances <5m (“contact events”, *e*)	62
Contact frequency (*e*/*d*=*f*)	0.0117
Proportion of nights when cattle are contacted all together (*g*; Table S4)	0.748
Proportion of nights when cattle are contacted independently (*h*; Table S4)	0.252
Proportion of nights when badgers contact cattle together (*i*; see text)	0 – 1*
Proportion of nights when badgers contact cattle independently (*j*; see text)	1 – 0*
Probability of collared badgers contacting any (collared & uncollared) cattle	
If cattle are all contacted together (*f*)	0.0117
If each of the cattle is contacted independently of other cattle ($1-(1-f)^{ab}$)	0.9799
Probability of any badger in a social group (collared & uncollared) contacting cattle	
<u>Scenario 1B: Badgers all move together and cattle are all contacted together</u>	
Probability that all the badgers in a social group contact cattle in this scenario (*f*)	0.0117
Average number of group contact events per night in this scenario (*f*)	0.0117
Proportion of nights when this scenario occurs (*i* x *g*=*k*1)	0.748 – 0
Proportion of nights with at least one contact of this type (*i* x *g* x *f* = *k*)	0.0087 – 0
<u>Scenario 2B: Badgers move independently and cattle are all contacted together</u>	
Probability that any badger in a group contacts cattle in this scenario ($1-(1-f)^{c}$)	0.0407
Average number of group contact events per night in this scenario (*c* x *f*)	0.041
Proportion of nights when this scenario occurs (*j* x *g* = *l*1)	0 – 0.748
Proportion of nights with at least one contact of this type ($j \times g \times [1-(1-f)^{c}] = l$)	0 – 0.0305
<u>Scenario 3B: Badgers all move together and cattle are contacted independently</u>	
Probability that all the badgers in a social group contact cattle in this scenario ($1-[1-f]^{ab}$)	0.9799
Average number of group contact events per night in this scenario (*a* x *b* x *f*)	3.886
Proportion of nights when this scenario occurs (*i* x *h* = *m*1)	0.2520 – 0
Proportion of nights with at least one contact of this type ($i \times h \times [1-(1-f)^{ab}] = m$)	0.2469 – 0
<u>Scenario 4B: Badgers move independently and cattle are contacted independently</u>	
Probability that any badger in a group contacts cattle in this scenario ($1-[(1-f)^{ab}]^{c}$)	1.000
Average number of group contact events per night in this scenario (*a* x *b* x *c* x *f*)	13.757
Proportion of nights when this scenario occurs (*j* x *h* = *n*1)	0 – 0.2520
Proportion of nights with at least one contact of this type ($j \times h \times [1-[(1-f)^{ab}]^{c}] = n$)	0 – 0.2520
Proportion of 24h periods with at least one contact event (k+l+m+n)	0.256-0.282
Average number of contact events per 24h period ([*f* x *k*1]+[*c* x *f* x *l*1]+[*a* x *b* x *f* x *m*1]+[*a* x *b* x *c* x *f* x *n*1])	0.988-3.498

*parameters *i* and *j* were not estimable so we considered the extreme cases of *i*=1,*j*=0 and *i*=0,*j*=1.

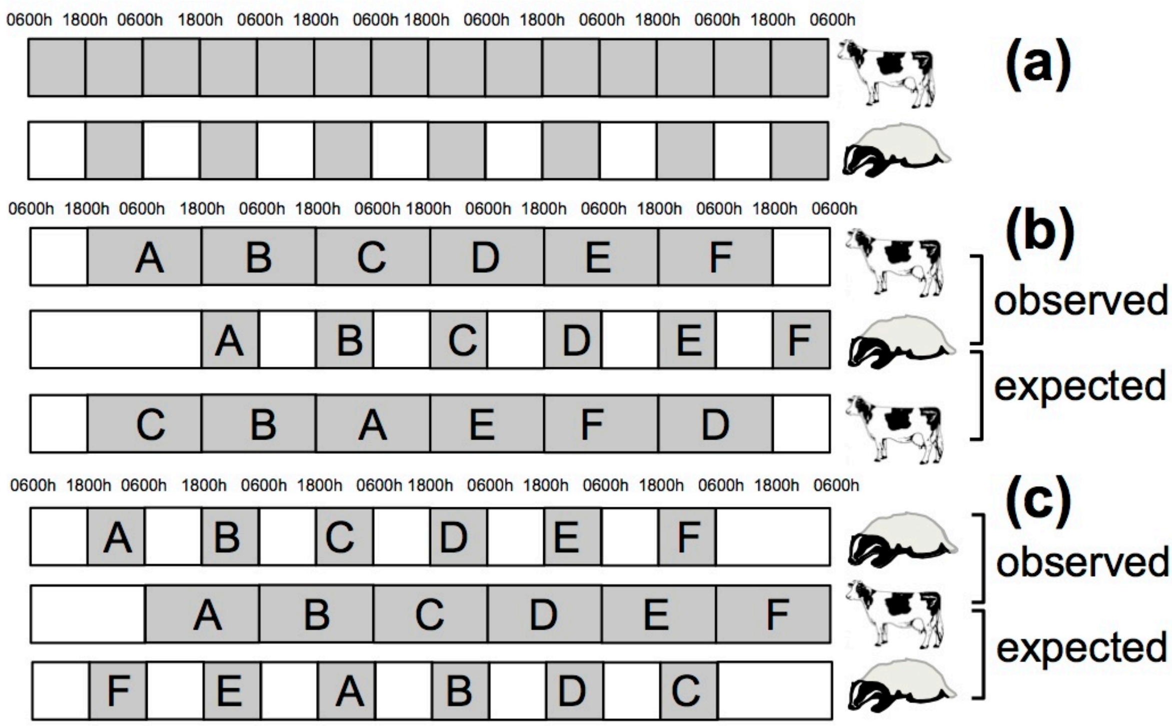

Figure 1 Comparison periods for badger and cattle locations. (a) Cattle collars recorded locations throughout each 24h-period, but badger collars attempted locations only for 12h periods between 1800h and 0600h. To explore potential indirect contact, we compared the badger locations observed each night with cattle locations from the preceding 24h-period (b), and the cattle locations observed in each 24h-period with badger locations from the previous night (c). The locations being compared were thus recorded a maximum of 36h apart. To explore potential avoidance, we generated "expected" movement patterns, comparing each night's badger locations with cattle locations from randomly-selected 24h-periods in the same tracking bout (b), and comparing cattle locations from each 24h-period with badger locations in the same field on randomly-selected nights during the same tracking bout (c).

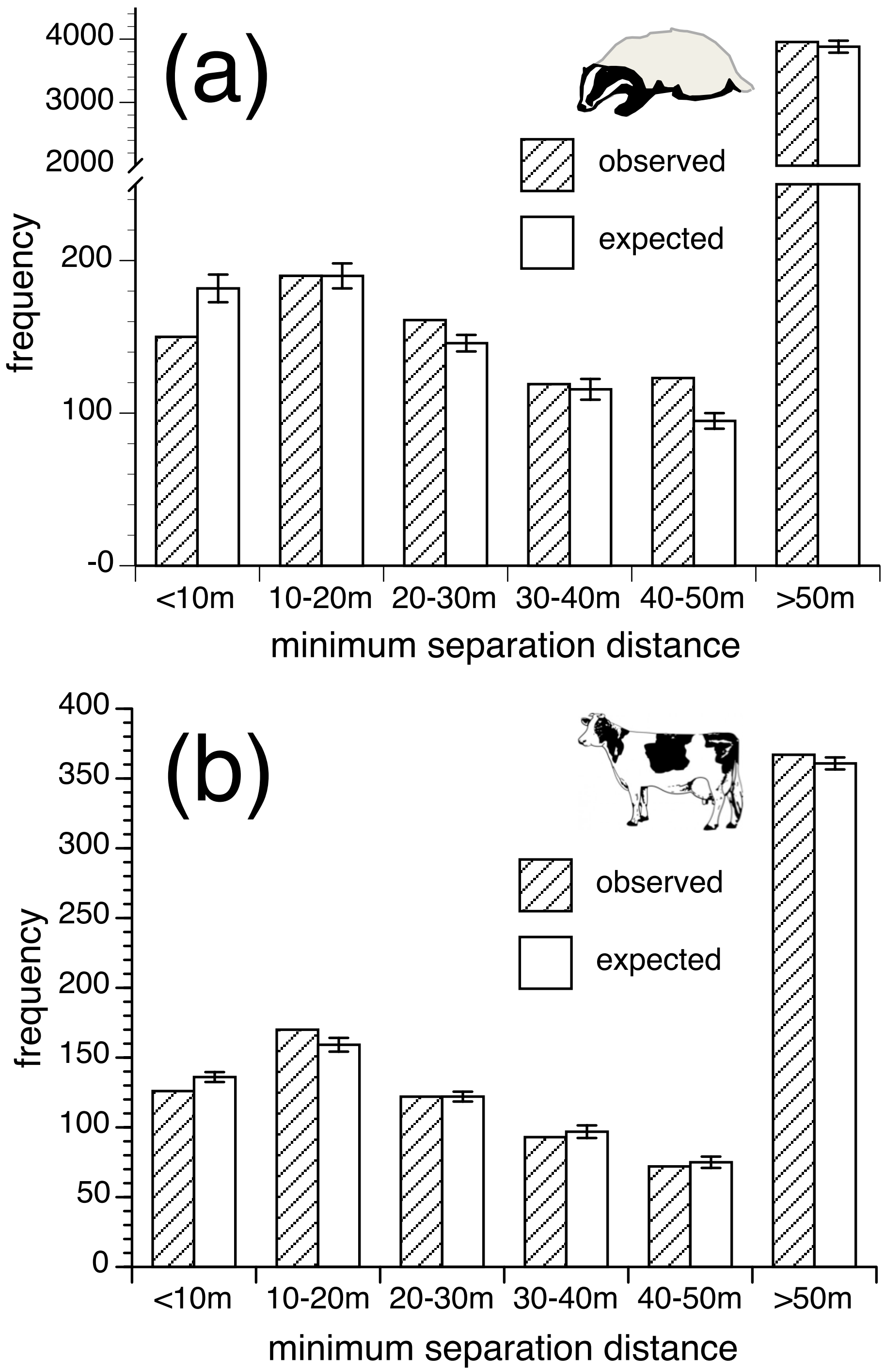

Figure 2 Observed and expected frequency distributions of minimum distances from (a) badgers, to cattle within the badgers' home ranges ≤36h previously; and (b) cattle, to badgers within the same field ≤36h previously. Expected distributions show the mean and 95% confidence interval from 20 permutations.

Successive use of shared space by badgers and cattle: implications for *Mycobacterium bovis* transmission

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SUPPLEMENTARY MATERIAL

1 Supplementary Methods

1.1 Filtering GPS-collar data

Following Woodroffe *et al.* (2016a), we filtered GPS-collar data from both species to remove potentially inaccurate locations. These filters were based upon error tests using stationary GPS collars; full details of these tests are provided in Woodroffe et al. (2016a). In summary, the filtering entailed excluding GPS-locations associated with <4 satellites and/or horizontal dilution of precision ≥4. We also excluded any badger locations >1km from both the preceding and subsequent locations, with 1km chosen as roughly twice the distance that a badger could cover in the 20mins between GPS-locations when travelling at the highest recorded speed for the species (Woodroffe et al. 2016a, Do Linh San, Ferrari & Weber 2007).

This filtering excluded 13% of cattle locations and 18% of badger locations. Sensitivity analyses have shown that this filtering improved location accuracy but did not bias analyses of habitat selection, building use, or ranging behaviour (Woodroffe et al. 2016a, Woodroffe *et al.* 2017, Woodroffe *et al.* 2016b). After filtering, GPS-collar locations were on average accurate to 4.7m (95% CI 4.5-4.9m) for badgers, and 4.6m (CI 4.5-4.7m) for cattle.

1.2 Avoidance of recently-occupied space outdoors only

As described in the main text, we used compositional analysis (Aebischer, Robertson & Kenward 1993) to test the hypothesis that badgers avoided space occupied by cattle ≤36hrs previously. This primary analysis used all cattle locations; however some collared cattle spent time inside farm buildings, and we have shown elsewhere that badgers avoided farm buildings on our study farms (Woodroffe et al. 2017). Avoidance of buildings could therefore generate spurious evidence of badgers avoiding cattle. To check whether the outcome of this analysis was affected by lack of badger access to buildings, we repeated the analysis excluding cattle locations ≤25m from farm buildings (the 25m cut-off was chosen for consistency with Woodroffe *et al.* (2017), and was based on location accuracy of the GPS-collar data (Woodroffe et al. 2016a)). This secondary analysis revealed no evidence of avoidance, a pattern similar to that obtained from the complete dataset (mean $p=0.445$, 95% CI 0.322-0.568).

2 Supplementary Tables

Table S1 – Survival times of *Mycobacterium bovis* in outdoor experiments

Location	Substrate	Detection method	Survival time	Source
Naturally infected samples				
Southern England	cow dung	guinea pig inoculation	1-4 months*	Williams & Hoy (1930)
	cow gut contents	guinea pig inoculation	<1 month	
Skukuza, South Africa	buffalo lung	culture	2-42 days†	Tanner & Michel (1999)
Artificially "spiked" samples				
Southern England	cow dung	guinea pig inoculation	2-5 months*	Williams & Hoy (1930)
New Zealand	dead possum	culture	2-27 days*	Barron *et al.* (2011)
Queensland, Australia	shaded soil	culture	4 weeks	Duffield & Young (1985)
New Zealand	cotton ribbons	culture	4-28 days†	Jackson, deLisle & Morris (1995)
Skukuza, South Africa	cattle dung	culture	2-28 days†	Tanner & Michel (1999)
Michigan, USA	hay	culture	<3-42 days**	Fine *et al.* (2011)
	soil	culture	8-63 days**	
	water	culture	12-32 days**	
	corn	culture	2-24 days**	
Michigan, USA	hay	PCR	8-10 months*	Adams *et al.* (2013)
	soil	PCR	7-8 months*	
	water	PCR	5-11+ months*	
	corn	PCR	0-9 months*	

*range across replicate samples; **range of means across seasons; †range across environmental conditions

Table S2 – Observed and expected frequency of **multiple cattle** contacting the **same badger**. The table shows the same data as Table S5, but counted as badger-24h-periods rather than badger-cattle-24h periods (e.g., two collared cattle tracked concurrently in the same 24h period after the same collared badger would count as two badger-cattle-24h periods in Table S5 but one badger-24h-period in this table). Data are shown separately for 24h periods when multiple collared cattle in the same herd had the opportunity to contact the same badger. Shading highlights values mentioned in the Main Text.

Collared cattle in a herd able to contact a focal collared badger	badger-24h-periods with minimum distance ≤5m												
	observed							*expected*					
	0	1	2	3	4	≥1	≥2	0	1	2	3	≥4	Total
1	750	6	–	–	–	6	–	*750**	*6**				756
2	499	10	1	–	–	10	1	501.9	8.0	0.0			510
3	298	13	0	0	–	13	0	303.7	7.3	0.1	0.0		311
4	167	3	0	0	0	3	0	164.7	5.3	0.1	0.0	0.0	170
5	145	4	1	0	0	5	1	144.1	5.8	0.1	0.0	0.0	150
6	116	7	1	0	1	9	2	119.2	5.7	0.1	0.0	0.0	125
7	58	2	1	1	0	4	2	58.6	3.3	0.1	0.0	0.0	62
8	49	2	1	1	0	4	2	49.7	3.2	0.1	0.0	0.0	53
9	11	0	0	0	0	0	0	10.2	0.7	0.0	0.0	0.0	11
10	2	0	0	0	0	0	0	1.8	0.1	0.0	0.0	0.0	2
11	1	0	0	0	0	0	0	0.9	0.1	0.0	0.0	0.0	1
Total	2,096	47	5	2	1	55	8	2,104.9	45.5	0.6	0.0	0.0	2,151
Total with >1 collared cattle	1,346	41	5	2	1	49	8	1,354.9	39.5	0.6	0.0	0.0	1,395

*These are observed values, used to calculate the expected values in the rows below them

Table S3 – Frequency of **cattle** contacting **multiple badgers**. The table reports the number of badger-cattle-24h-periods on which collared cattle were detected ≤5m from locations where collared badgers had been detected in the previous ≤36h. Data are shown separately for 24h periods when there were multiple collared badgers in potential contact with the same cattle.

Number of badgers matched with the collared bovine being tracked the previous night	number of badger-cattle-24h-periods with minimum distance ≤5m			
	0	1	2	Total
1	2,439	24	–	2,463
2	2,525	29	6	2,560
3	772	8	0	780
4	64	0	0	64
5	10	0	0	10
Total	5,810	61	6	5,877

Table S4 – Models of the probability of indirect contact with multiple collared animals in the same cattle group or badger social group. For mixture models, the percentages indicate the best-fit estimates. Shading indicates the best-fit models.

	Assumptions about how multiple collared individuals of the other species are contacted		
	Contacted independently	*Mixture of independent & clustered contacts*	*Mixture of independent & avoided contacts*
Cattle contacting badgers			
Log Likelihood	-410.1	-389.1	-410.1
% independent	–	61.9%	100%
% together	–	38.1%	0%
Badgers contacting cattle			
Log Likelihood	-531.2	-435.6	-533.1
% independent	–	25.2%	73.6%
% together	–	74.8%	26.4%

Table S5 – Frequency of **multiple cattle** contacting the **same badger**. The table reports the number of badger-cattle-24h periods on which collared cattle were detected ≤5m from locations where collared badgers had been detected in the previous ≤36h. Data are shown separately for 24h periods when multiple collared cattle in the same herd had the opportunity to contact the same badger.

Number of collared cattle in a herd which could contact a particular collared badger	number of badger-cattle-24h-periods with minimum distance ≤5m					
	0	1	2	3	4	Total
1	750	6	–	–	–	756
2	1,008	10	2	–	–	1,020
3	920	13	0	0	–	933
4	677	3	0	0	0	680
5	744	4	2	0	0	750
6	737	7	2	0	4	750
7	427	2	2	3	0	434
8	417	2	2	3	0	424
9	99	0	0	0	0	99
10	20	0	0	0	0	20
11	11	0	0	0	0	11
Total	5,810	47	10	6	4	5,877

Table S6 – Sensitivity analysis of the estimated frequency of **cattle contacting badgers**. To explore the robustness of our estimates of contact frequency, we repeated our calculations of the average number of contacts per 24h period using the upper and lower 95% confidence limit for each parameter (F, G, I). Sensitivity to estimates of cattle herd size and badger social group size was not investigated, because a larger herd would be likely to occupy a larger area and hence could potentially encounter more social groups of badgers, by an amount not estimated in this study. These calculations can be reproduced using Spreadsheet S2.

	Parameter estimates			**Contacts per 24h**		
Parameter	*baseline*	*lower*	*upper*	*baseline*	*lower*	*upper*
Proportion of badger-cattle-24h-periods which include a contact event (F)	0.0114	0.0088	0.0145	7.727	5.965	9.828
Proportion of 24h periods when badgers are contacted all together (G)	0.381	0.214	0.541	7.727	6.238	9.280
Proportion of 24h periods when herd members contact badgers together (I)	0.0204	0.0005	0.109	7.727	7.032	7.883

Table S7 – Frequency of a **badger** contacting **multiple cattle**. The table reports the number of cattle-badger-nights on which collared badgers were detected ≤5m from locations where collared cattle had been detected in the previous ≤36h. Data are shown separately for nights when there were multiple collared cattle in potential contact with the same badger.

Number of cattle matched with the collared badger tracked the previous 24h	number of cattle-badger-nights with minimum distance ≤5m				
	0	1	2	3	Total
1	541	5	–	–	546
2	888	14	0	–	902
3	547	14	0	0	561
4	683	9	0	0	692
5	614	4	2	0	620
6	585	3	0	0	588
7	421	1	2	3	427
8	523	5	0	0	528
9	234	0	0	0	234
10	120	0	0	0	120
11	0	0	0	0	0
12	36	0	0	0	36
13	39	0	0	0	39
14	14	0	0	0	14
Total	5,245	55	4	3	5,307

Table S8 – Frequency of **multiple badgers** contacting the **same cattle**. The table reports the number of cattle-badger-nights on which collared badgers were detected ≤5m from locations where collared cattle had been detected in the previous ≤36h. Data are shown separately for nights when multiple collared badgers in the same social group had the opportunity to contact the same cattle.

Collared badgers in the same social group which could potentially contact the same collared cattle	number of cattle-badger-nights with minimum distance ≤5m			
	0	1	2	Total
1	4,410	37	–	4,447
2	835	25	0	860
Total	5,245	62	0	5,307

Table S9 – Observed and expected frequency of **multiple badgers** contacting the **same cattle**. The table shows the same data as Table S7, but counted as cattle-nights rather than cattle-badger-nights periods on which (e.g., two collared badgers tracked concurrently on the same night after the same collared cow would count as two cattle- badger-nights in Table S7 but one cattle-night in this table). Data are shown separately for nights when multiple collared badgers in the same social group had the opportunity to contact the same cattle.

Collared badgers in the same social group potentially able to contact the same collared cattle	cattle-nights with minimum distance ≤5m						
	observed			*expected*			
	0	1	2	0	1	2	Total
1	4,410	37	–	4,410.00	37.00	–	4,447
2	405	25	0	422.87	7.10	0.03	430
Total	4,815	62	0	4,832.87	44.10	0.03	4,877

Table S10 – Sensitivity analysis of the estimated frequency of **badgers contacting cattle**. To explore the robustness of our estimates of contact frequency, we repeated our calculations of the average number of contacts per night using the upper and lower 95% confidence limits for parameters *f* and *g*, and the full range of parameter *i* (a proportion which could not be estimated and so was represented as either 1 or 0). Sensitivity to estimates of badger social group size and cattle herd size was not investigated, because a larger social group would be likely to occupy a smaller area (as higher badger densities are typified by larger groups and smaller territories) and hence could potentially encounter fewer cattle herds, by an amount not estimated in this study. These calculations can be reproduced using Spreadsheet S2.

Parameter	**Parameter estimates**			**Contacts per night**		
	baseline	*lower 95%*	*upper 95%*	*baseline*	*lower*	*upper*
When badgers always contact cattle independently (i=0)						
Proportion of cattle-badger-nights which include a contact event (*f*)	0.0117	0.009	0.015	3.498	2.695	4.491
Proportion of nights when cattle are contacted all together (*g*)	0.748	0.678	0.808	3.498	2.675	4.458
When badgers always contact cattle together (i=1)						
Proportion of cattle-badger-nights which include a contact event (*f*)	0.0117	0.009	0.015	0.988	0.761	1.269
Proportion of nights when cattle are contacted all together (*g*)	0.748	0.678	0.808	0.988	0.756	1.259

3 Supplementary Figures

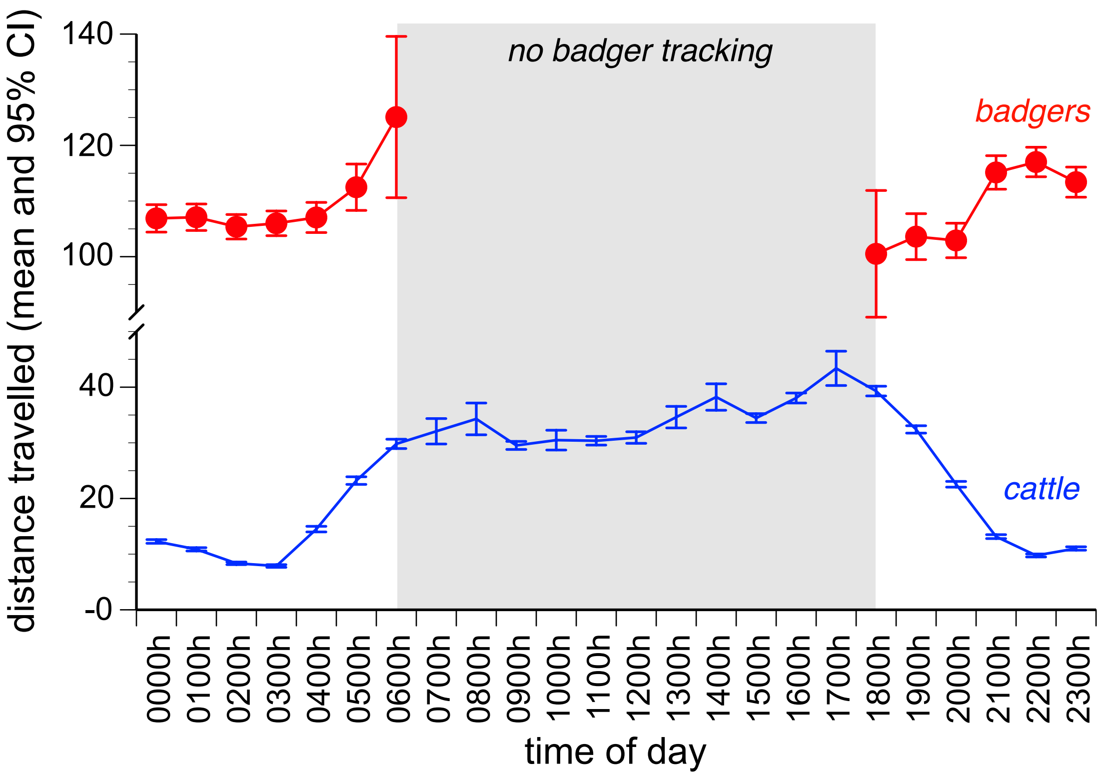

Figure S1 – Diel variation in movement behaviour of 421 cattle (blue) and 54 badgers (red), measured as the straight-line distance travelled (in metres) between successive GPS-collar locations collected 20 mins apart. As badgers are nocturnal, they were only monitored while active, and between the hours of 1800h and 0600h UTC. Cattle were monitored for 24h per day. This graph does not account for any seasonal variation in activity patterns.

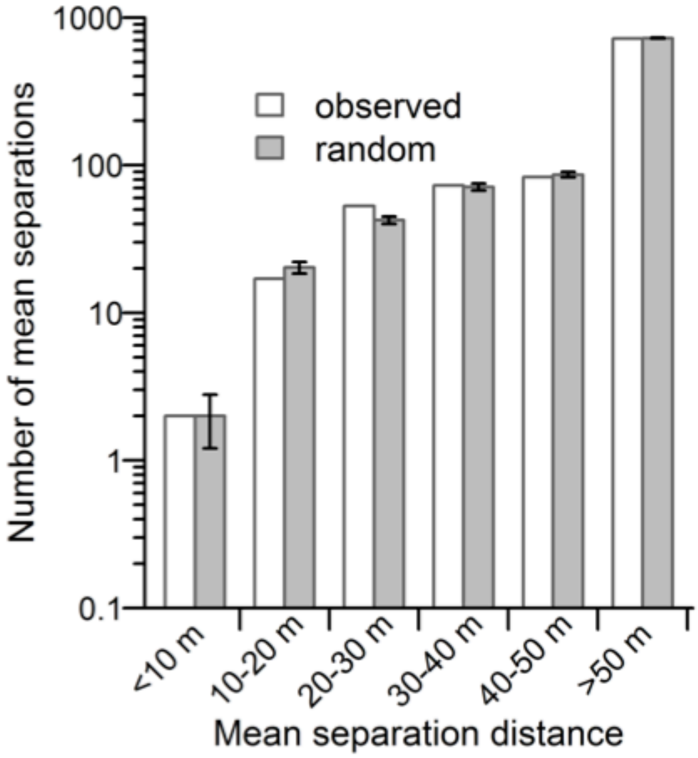

Figure S2 – Frequency distribution of mean separation distances between individual cattle during 24h tracking periods (0600h-0600h), and individual badgers located in the same fields during the immediately preceding night (1800h-0600h). This graph is equivalent to Figure 2(b) in the main text, and uses the same 950 badger-cattle-24h-periods of tracking, but presents the mean distance between individual badger-cattle pairs rather than the minimum. The graph compares the observed distribution of mean separation distances between pairs of individual cattle and badgers with the mean and 95% CI from 20 permutations linking each 24h-period of cattle tracking with a different, randomly-selected, night of tracking the same paired badger.

4 References

Adams, A. P., S. R. Bolin, A. E. Fine, C. A. Bolin & J. B. Kaneene (2013) Comparison of PCR versus culture for detection of *Mycobacterium bovis* after experimental inoculation of various matrices held under environmental conditions for extended periods. *Applied and Environmental Microbiology,* **79,** 6501-6506.

Aebischer, N. J., P. A. Robertson & R. E. Kenward (1993) Compositional analysis of habitat use from animal radio-tracking data. *Ecology,* **74,** 1313-1325.

Barron, M. C., R. P. Pech, J. Whitford, I. J. Yockney, G. W. de Lisle & G. Nugent (2011) Longevity of *Mycobacterium bovis* in brushtail possum (*Trichosurus vulpecula*) carcasses, and contact rates between possums and carcasses. *New Zealand Veterinary Journal,* **59,** 209-217.

Do Linh San, E., N. Ferrari & J. M. Weber (2007) Spatio-temporal ecology and density of badgers *Meles meles* in the Swiss Jura Mountains. *European Journal of Wildlife Research,* **53,** 265-275.

Duffield, B. J. & D. A. Young (1985) Survival of *Mycobacterium bovis* in defined environmental conditions. *Veterinary Microbiology,* **10,** 193-197.

Fine, A. E., C. A. Bolin, J. C. Gardiner & J. B. Kaneene (2011) A study of the persistence of *Mycobacterium bovis* in the environment under natural weather conditions in Michigan, USA. *Veterinary medicine international,* **2011,** 765430-765430.

Jackson, R., G. W. deLisle & R. S. Morris (1995) A study of the environmental survival of *Mycobacterium bovis* on a farm in New Zealand. *New Zealand Veterinary Journal,* **43,** 346-352.

Tanner, M. & A. L. Michel (1999) Investigation of the viability of *M. bovis* under different environmental conditions in the Kruger National Park. *Onderstepoort Journal of Veterinary Research,* **66,** 185-190.

Williams, R. S. & W. A. Hoy (1930) The viability of *B. tuberculosis* (*bovinus*) on pasture land, in stored faeces and in liquid manure. *Journal of Hygiene,* **30,** 413-419.

Woodroffe, R., C. A. Donnelly, C. Ham, S. Y. B. Jackson, K. Moyes, K. Chapman, N. G. Stratton & S. J. Cartwright (2016a) Badgers prefer cattle pasture but avoid cattle: implications for bovine tuberculosis control. *Ecology Letters,* **19,** 1201-1208.

Woodroffe, R., C. A. Donnelly, C. Ham, S. Y. B. Jackson, K. Moyes, K. Chapman, N. G. Stratton & S. J. Cartwright (2016b) Ranging behaviour of badgers *Meles meles* L. vaccinated with Bacillus Calmette Guerin. *Journal of Applied Ecology,* **54,** 718-725.

Woodroffe, R., C. A. Donnelly, C. Ham, S. Y. B. Jackson, K. Moyes, K. Chapman, N. G. Stratton & S. J. Cartwright (2017) Use of farm buildings by wild badgers: implications for the transmission of bovine tuberculosis. *European Journal of Wildlife Research,* **63,** 6.