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Patterns of direct and indirect contact between cattle and badgers naturally infected with tuberculosis

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SUMMARY

Tuberculosis (TB) due to infection with *Mycobacterium bovis* is transmitted between cattle and badgers (*Meles meles*) in the UK and Ireland but it is unclear where or when transmission occurs. We investigated direct and indirect interactions between badgers and cattle using automated proximity loggers on animals and at badger latrines located on pasture, in an area of south-west England with a high-density badger population. Direct contacts (interactions within 1·4 m) between badgers and cattle at pasture were very rare (four out of >500 000 recorded animal-to-animal contacts) despite ample opportunity for interactions to occur. Indirect interactions (visits to badger latrines by badgers and cattle) were two orders of magnitude more frequent than direct contacts: 400 visits by badgers and 1700 visits by cattle were recorded. This suggests that indirect contacts might be more important than direct contacts in terms of disease transmission at pasture. The TB infection status of individual badgers (ascribed with 93% accuracy using three diagnostic tests) did not affect the frequency or duration of their visits to latrines located on pasture grazed by cattle. Nevertheless, there was wide variation in contact behaviour between individuals, which highlights the importance of understanding heterogeneity in contact patterns when developing strategies to control disease spread in wildlife and livestock.

Key words: Disease control, livestock, *Mycobacterium bovis*, wildlife.

INTRODUCTION

Bovine tuberculosis (TB) caused by infection with *Mycobacterium bovis* is endemic in cattle in parts of the UK and Ireland and its control is hindered by the presence of infection in the European badger (*Meles meles*). While *M. bovis* is clearly transmitted between cattle and badgers [1], it is not known where, when or how often transmission occurs. The principal route of transmission from badgers to cattle is unclear although transfer of infection has been demonstrated when captive badgers and cattle have been kept in close proximity [2]. Aerosol transmission is thought to be the main route for *M. bovis* transfer between badgers and cattle [3] although infectious badgers can excrete *M. bovis* bacilli in faeces, urine, sputum and exudate from wounds and abscesses [4]. Thus, badger latrines on pasture represent a potential

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source of infection for cattle [5]. Latrines are clusters of shallow pits in the ground into which badgers defecate. In addition, badgers use these communal latrines to demarcate territorial boundaries, and so they are likely to represent nodes of interaction among individuals from neighbouring social groups [6]. In summary, interactions with badgers (direct contacts) or their excretions (indirect contacts) may present opportunities for the infection of cattle via inhalation and ingestion [7, 8].

Demonstrating routes of transmission in the field would be technically and ethically challenging and hence risks of transmission need to be inferred from descriptions of direct and indirect contacts between badgers and cattle. Observational field studies to investigate interactions between these two species at pasture have recorded relatively infrequent direct contact [9]. However, recording interaction data manually is time-consuming and labour-intensive, and consequently only small numbers of animals can be observed this way. One solution is to use proximity loggers: remote-sensing devices that transmit a unique signal and automatically record the frequency and duration of contacts when tagged animals come within a pre-set distance of one another. The use of proximity loggers in recording interactions between badgers and cattle has recently been validated to confirm the accuracy and reliability of the data [10]. This technology has also been used to record interactions between a small number of animals (11 badgers and 13 cattle) by Böhm *et al.* [11], but the TB infection status of these animals was not measured.

The probability of contact between badgers and cattle is influenced by individual behaviour [11]. Observations of grazing cattle show variation in the kind of exploratory behaviour which may bring them into contact with badgers or their excretory products [12, 13]. Such traits may be linked to age, sex, breed or individual behaviour patterns. Furthermore, badger movement behaviour varies among individuals, relative to age, sex [14] and infection status [15]. Long-term monitoring of *M. bovis* dynamics in a badger population at Woodchester Park in south-west England suggests that individuals known to be excreting *M. bovis* may have larger home ranges and be more likely to visit farm buildings [15] and seropositivity has been associated with differential denning behaviour [16]. Understanding the behaviour of infectious individuals can be of fundamental importance in managing disease because targeted control efforts (whereby high-risk individuals are prophylactically vaccinated, treated or culled) can be substantially more effective at preventing and containing outbreaks than generalized approaches [17, 18]. Even more effective control may be possible if predictions can be made to identify accurately individuals (or groups) with the potential for disproportionate contribution to transmission before they infect others, for example based on individual or group characteristics such as age or sex [19]. In order to develop and optimize effective strategies for targeted disease control, research efforts need to be focused on the identification of easily recognizable host or environmental traits associated with high transmission risks [20].

The aim of the present study was to quantify rates of direct (animal-to-animal) and indirect (animal-to-latrine) contact between badgers and cattle at pasture in an area of high-badger density where the prevalence of TB in badgers and the incidence of TB in cattle are both high. Our study aimed to identify risk factors for contacts and to describe individual-level variation in behaviour, and hence generate information of value in developing disease control strategies for TB in badgers and cattle, while contributing to our broader understanding of infectious disease control.

MATERIALS AND METHODS

Study design and study area

The study was conducted from September 2009 to August 2010 at Woodchester Park, Gloucestershire, UK (51° 43′ N, 2° 16 W). The study area comprises a 7 km^2 region of Cotswold limestone escarpment consisting of a valley with areas of pasture grazed by cattle and flanked by mixed woodland (Fig. 1). An intensively studied high-density population of about 215 wild badgers (population size in 2009 estimated by the minimum number alive method [21]) distributed in 23 social groups live within the study area. This badger population has been the subject of long-term research into badger ecology and TB epidemiology [22].

Study animals

Cattle

All 28 animals present at the start of the study in a herd of Welsh Black cattle were fitted with proximity logger collars (see below for logger details). Five newborn calves were also fitted with collars in June 2010, resulting in contact data being recorded from a total of 33 animals for up to 12 months. Cattle were aged

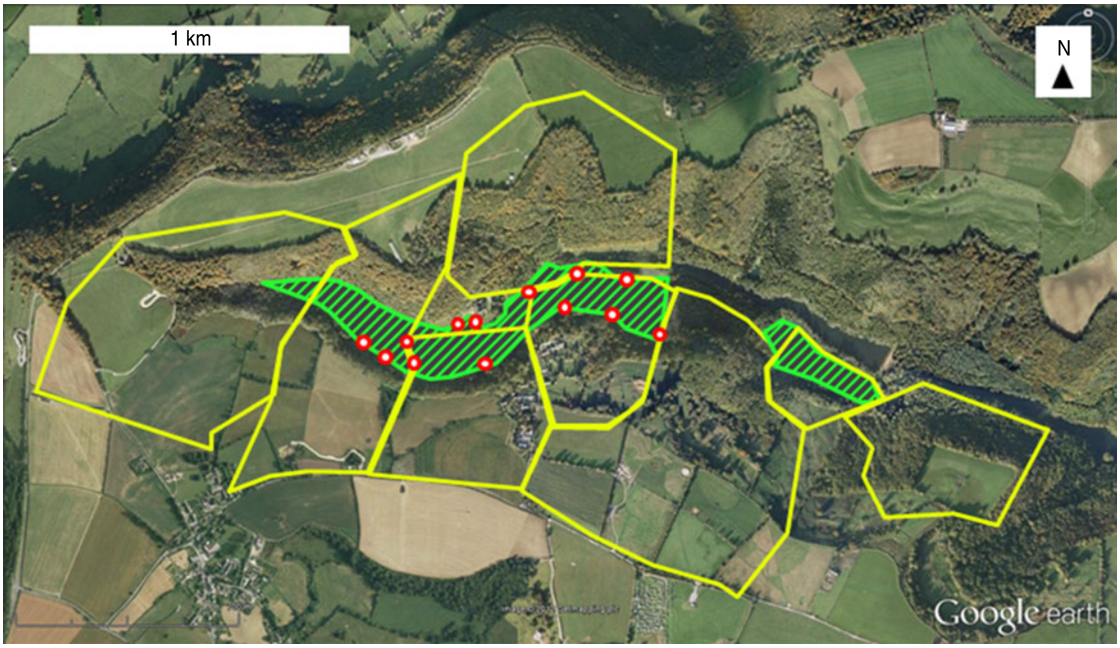

Fig. 1. [*colour online*]. Map of the study area showing the overlap between badger social group territories (yellow polygons) and fields grazed by cattle (green hatching). The locations of active badger latrines where proximity logger base stations were placed are indicated by red circles. (Map image © 2012 Google and © 2012 Getmapping plc.)

between <1 and 136 (median 38) months on entering the study. Each of the 24 female and nine male animals was identified by its unique ear tag number.

Badgers

Proximity loggers were also fitted to 61 badgers (32 male, 29 female) from the eight social groups whose territories surrounded the pasture grazed by the collared cattle (see Fig. 1). This represented about 75% of the badgers estimated to be resident in these groups (figures derived from a capture-mark-recapture study: see [22] for details of methods). An almost equal number of adults ($n = 31$) and yearlings (those aged between 1 and 2 years, $n = 30$) were collared. Badgers were trapped at active setts (burrow systems) using steel mesh box traps [23] and sampled under anaesthesia at time of collaring to ascertain their TB infection status. TB status was determined using parallel interpretation of three diagnostic tests: mycobacterial culture of clinical samples (tracheal and oesophageal aspirates, urine, faeces and wound swabs) [4]; a gamma-interferon assay (a measure of T-cell immunity) [24]; and a serological assay to examine for IgM and IgG antibodies to a cocktail of *M. tuberculosis*-complex antigens (Stat-Pak, Chembio Diagnostic Systems, USA) [25]. This approach is estimated to have an overall diagnostic accuracy at the individual badger level of 93% (range 75–97%) [26], and in this instance identified 23 (38%) of the collared badgers in the present study as TB-positive (15 adults and eight yearlings).

Proximity loggers

Proximity loggers (Sirtrack Tracking Solutions, New Zealand) were mounted on neck collars (made of leather for badgers and nylon for cattle) and fitted to the cattle and badgers. Full details of the loggers and their operation can be found in a previous publication [10]. Badger collars were set to detect other loggers coming within 0·4–1·4 m and cattle collars had detection distances of 1·5–1·9 m (although only records reciprocally recorded on both cattle and badger collars were used in the analysis of interspecies interactions, representing a maximum interspecies distance of 1·4 m for recorded contacts). This distance is likely to be biologically meaningful in the epidemiology of TB because it approximates the 1·5–2·0 m aerosol transmission distance postulated to occur between cattle and other animals [27].

In addition to the proximity loggers fitted to animals, 19 static base stations were buried in plastic tubes in the ground next to badger latrines. Latrines were identified from a systematic survey carried out earlier in 2009 after a bait-marking exercise. This involved feeding a bait of peanuts and syrup mixed with indigestible plastic beads at all the active main setts in the study area for 10 days. Each sett was fed bait with a uniquely coloured or shaped plastic marker so that when these were found in droppings during a subsequent systematic survey for latrines, the territory boundaries of each social group could be identified (for further details see [28, 29]). Latrines were individually classified as being either on the boundary or in the hinterland of badger social group territories, a distinction that reflects their differing functions in territorial defence and communication [6]. Boundary latrines were classified as those within 5 m of the social group boundary, whereas hinterland latrines were further away.

A proximity logger base station was deployed at each active latrine in the three main fields to which the grazing cattle had access (Fig. 1). The base stations were deployed for 6 months during the second half of the study (April–September 2010). Base stations were set to detect the proximity loggers worn by badgers or cattle coming within a horizontal distance at ground level of 0·3–0·8 m from the latrine. Field trials showed this setting resulted in base stations having a greater detection distance above ground level than did proximity loggers worn by badgers at ground level, due to the effect of ground absorption of radio waves [10]. Because of this difference in detection capability, some contacts were not reciprocally recorded by both loggers in an interacting pair and so this part of the analysis was conducted only on the data recorded by the base stations.

Data cleaning

In order to reduce bias resulting from incomplete recording of long-duration contacts and loggers partially interacting at the edge of their detection range, the raw data derived from all three types of proximity logger were cleaned by amalgamating records within a rolling 60-s time-frame and removing any remaining 1-s records. This method has been shown to result in data that reflect observed interaction patterns in badgers and cattle [10].

Regression model

In order to determine the risk factors for visits by badgers to latrines on pasture, survival analysis on the recorded proximity data was conducted using a Cox proportional hazards model stratified by calendar month. An event was classified as a visit by a cow or badger to a marked latrine. The time until a latrine visit occurred was modelled allowing for multiple visits per animal. Each of the following badger covariates was assessed using the partial likelihood ratio test with a cut-off of $P<0·07$ for inclusion in the multivariable model [30]: *M. bovis*-infection status (positive or negative) on the date of entry to the study; age group (adult or yearling); and sex. All variables were retained in the final model, with the addition of badger social group identity to account for the uneven number of base stations in each group's territory. The regression analyses were run using Stata statistical software version 12 (StataCorp., USA).

RESULTS

Direct contacts between cattle and badgers

Only four direct contacts between cattle and badgers were recorded during the 12-month study: two in March, one in April and one in October. This compares with 382332 records of interactions between cattle, and 200199 records of interactions between badgers (a total of 582535 animal-to-animal contacts). The four interspecies interactions involved three cattle (9% of the herd) and three badgers (5% of collared individuals). The ages of the three cattle involved in interspecies interactions were: 4 months, 11 months, and 5 years. The badgers comprised one adult and two yearlings. Two of these three badgers tested positive for TB. Each interaction lasted between 4 s and 12 s (median duration 10 s). All interactions occurred at night, the earliest after sunset at 21:39 hours (in October) and the latest at 01:16 hours (in April).

Indirect contacts between cattle and badgers

Six of the 19 base stations deployed (32%) were lost during the study (thought most likely to have been dug up and removed by badgers) and so the analysis of indirect interactions was limited to visits by badgers and cattle to the 13 latrines for which data were available.

Badger visits to latrines

In total, there were 383 records of badger visits to latrines located on the pasture grazed by cattle. These visits were made by 21 badgers (34% of collared individuals) and the majority of visits (376/383) took place at night between 21:00 and 05:00 hours

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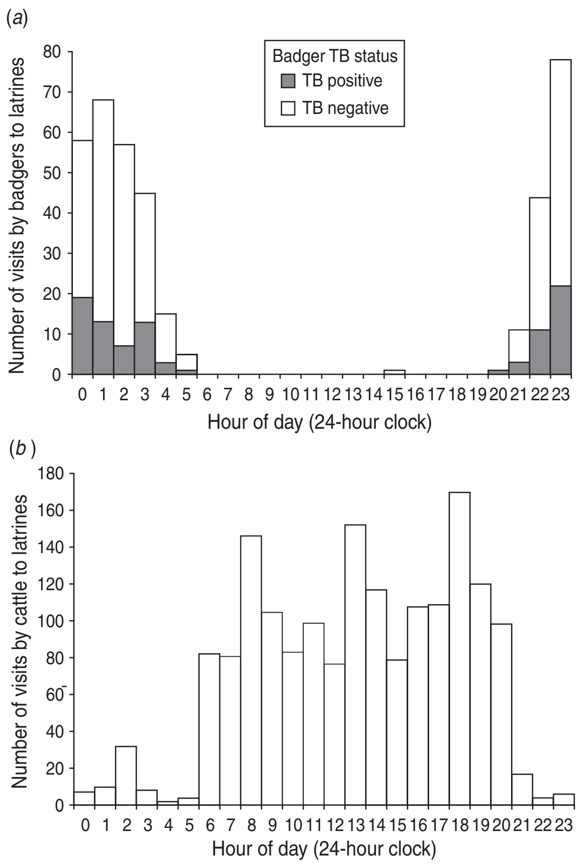

Fig. 2. Timing of visits by (*a*) badgers and (*b*) cattle to 13 active badger latrines located on pasture. The data summarize the 383 visits to latrines made by 21 badgers (out of 61 fitted with proximity loggers, of which 23 tested TB-positive and 38 tested TB-negative) and 1716 visits to latrines made by 28 cattle (out of 33 fitted with proximity loggers) from April to September 2010.

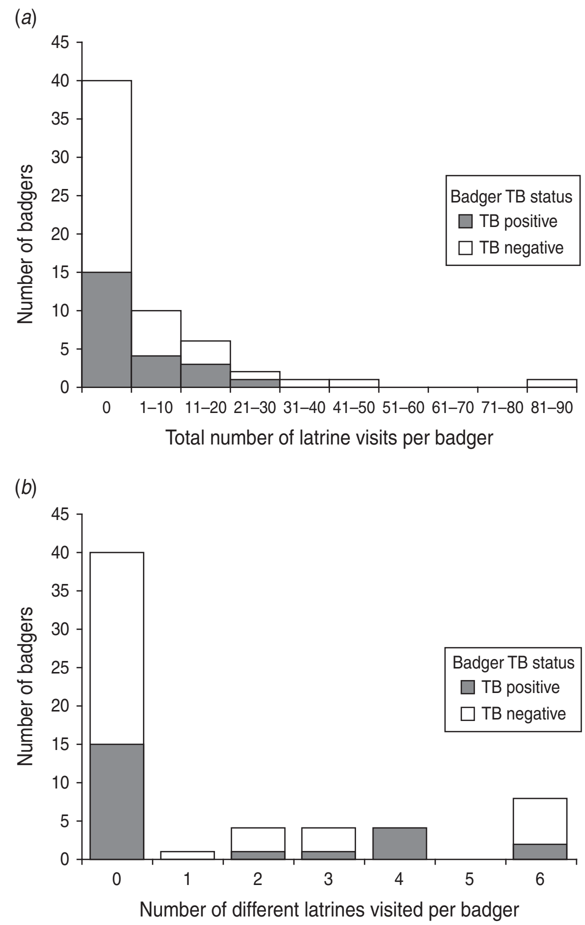

Fig. 3. Distribution of badger visits to latrines located on cattle pasture. (*a*) Number of visits to latrines made by each badger; (*b*) number of different latrines visited by each badger. No single badger visited more than six different latrines. The data summarize the 383 visits to 13 latrines by 61 badgers (of which 23 tested TB-positive and 38 tested TB-negative) recorded from April to September 2010.

(Fig. 2*a*). We found evidence of behavioural heterogeneity in badger visits to latrines, in respect of the total number of latrine visits made by individuals and the number of different latrines visited by each badger. Most badgers made no visits or only a small number of visits to the latrines monitored by base stations, whereas a few individuals made many visits (Fig. 3*a*). Of those badgers that visited the latrines with proximity to base stations, the median number of visits per individual was two per month (range 0–43 latrine visits per badger per month). The median duration of each latrine visit was 6 s (range 2–70 s). These visits were distributed across all 13 of the recovered base stations, although no single badger visited more than six of the latrines with a base station during the course of the study (Fig. 3b).

Adult badgers were less likely than yearlings to visit latrines on pasture (hazard ratio 0·446, 95% confidence interval 0·269–0·738, $P = 0{\cdot}002$) but there was no effect of sex or *M. bovis* infection status on the likelihood of an individual badger visiting latrines. No significant differences were found in the frequency or duration of visits by TB-positive and TB-negative badgers to latrines, nor was there any difference between the likelihood of TB-positive and TB-negative badgers visiting latrines at territory boundaries or in the hinterland (χ^2 tests with Yates' correction, $P > 0{\cdot}05$).

Cattle visits to badger latrines

There were 1716 records of cattle visiting base stations at badger latrines. These visits involved 28 cattle (85% of the herd). The median duration that cattle spent at each latrine was 31 s (range 2–1273 s) suggesting that

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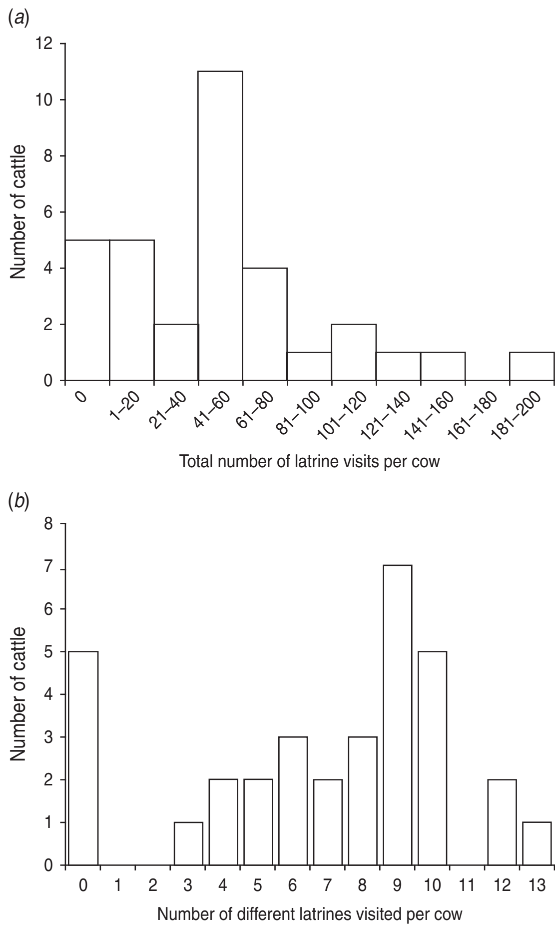

Fig. 4. Distribution of cattle visits to 13 badger latrines located on pasture. (*a*) Number of visits to latrines made by each cow; (*b*) number of different latrines visited by each cow. A single cow visited all 13 latrines. The data summarize the 1716 visits to active badger latrines recorded by proximity loggers fitted to all members of a herd of 33 cattle from April to September 2010.

the majority of records was likely to have resulted from individuals actively investigating the latrines, or grazing right next to them, rather than simply walking past. Most cattle made fewer than 50 visits to badger latrines, although a small proportion made many more: five of the 33 cattle made >100 visits each, including one animal which made 197 visits (Fig. 4*a*). Cattle visits to latrines were distributed across all 13 of the recovered base stations, with most animals visiting nine latrines and one visiting all 13 (Fig. 4*b*). Unlike the visits made by badgers, most visits to latrines by cattle occurred during the day (Fig. 2*b*).

DISCUSSION

This study used automated proximity loggers fitted to individual animals and static base stations located at badger latrines to determine patterns of direct and indirect contacts between badgers and cattle at pasture. This research builds on that conducted by Böhm *et al.* [11] on interactions between badgers and cattle at pasture. In the present study we were able to assemble a larger number of study animals, with supporting data on TB infection status, and to examine indirect contacts at latrines. Consequently the present study has provided estimates for rates of direct badger–cattle contact and visits to badger latrines (potential sources of infection) by both species, which may be useful for parameterizing epidemiological models of TB transmission used to inform disease control programmes.

Our findings suggest that direct contacts between badgers and cattle occur very rarely at pasture. This corroborates the results of previous studies based on direct observations [9] and proximity-logger records [11]. In our study only four direct interactions were recorded during a 12-month period despite being in an area of high badger density. This low number of contacts meant that it was not possible to assess the effect of TB status on interspecies interactions.

The low contact rate between cattle and badgers suggests that the probability of TB transmission from direct contacts between the species at pasture may be small, although the consequence of such an exposure could be large. However, this study did not provide information on the likelihood of transmission at any given contact event, conditional on one host being infectious. An unbroken chain of events is necessary for a given contact to result in pathogen transmission: in order for cattle to become infected from contact with a badger, not only must the latter be excreting *M. bovis* at that time, but the excreted organisms must be available in a sufficient dose, and the cattle must behave in such a way as to encounter the organism [31]. Therefore, to assess the risk of transmission of *M. bovis* to cattle from infectious badgers, several factors must be addressed, including the susceptibility of cattle to each of the possible routes of infection (principally inhalation and less so ingestion), the minimum infectious dose for each route (low for inhalation, high for ingestion), and the probability that cattle will encounter an infectious dose by that route [31]. The difficulties of accurately diagnosing TB in live animals mean it is almost impossible to measure transmission events in a non-experimental setting, and hence relative risks of transmission need to be inferred from data on direct and indirect contact behaviour between badgers and cattle, such as those generated by the present study.

Indirect contacts (visits by both species to badger latrines) were more common than direct contacts between badgers and cattle by two orders of magnitude (hundreds of latrine visits were recorded), suggesting that these might represent the more typical nature of interspecies contact. These findings suggest that future studies aiming to quantify the exposure of cattle to tuberculous badgers might usefully focus on indirect contacts with badgers at latrines or on direct or indirect contacts with badgers occurring elsewhere, for example in farm buildings [32].

A limitation of the present study was that proximity base stations were not deployed for the entire 12 months. Visits to latrines were recorded for 6 months, from April to September, which means that they were not monitored over the entire periods of peak badger activity in spring and autumn [33]. It is likely that badgers also used latrines that were not observed because only the latrines on pasture were monitored by base stations. This could have underestimated the number of visits and hence the potential number of indirect interactions, further emphasizing the magnitude of the difference in frequency between direct and indirect contacts. However, this would not have affected the measurement of direct contacts with cattle since the proximity loggers on collars were deployed throughout all seasons.

Despite the low direct contact rate recorded between badgers and cattle in the present study, it is probable that such contact will be even less frequent in areas of the country where there are fewer badgers. Furthermore, local landscape composition and configuration could have a profound influence on contact rates through effects on badger foraging patterns. The pasture occupied by the cattle herd in the present study was frequently used by foraging badgers and is completely surrounded by woodland (where badger setts were located) which is likely to have increased chances of contact between the two species. Hence results from the present study suggest that the two species may be ignoring or even actively avoiding one another. Such a high level of overlap between the species is unlikely to exist on many farms, meaning direct contact between cattle and badgers at pasture could be very rare. Variation in farming practices (including beef *vs.* dairy cattle) was not explored in the present study which focused on only one farm. The contact rates identified in the present study should therefore be taken as an indication of relative exposures in similar farms in the same region of England rather than a definitive estimation.

The present study showed that adult badgers were less likely than yearlings to visit latrines on pasture. While this suggests that cattle were more likely to be exposed to yearling badgers, the prevalence of *M. bovis* infection is higher in adult badgers [34]. Thus the potential relative infection risk to cattle from yearling or adult badgers is not clear. A pressing area for future research would be to examine the relative contribution to disease transmission of individuals involved in the highest frequency of interactions ('supercontactors') with that of the most infectious individuals ('supershedders' or 'superexcretors') and to identify the attributes of individuals which fall into both categories for disease transmission ('superspreaders'). Targeting this latter group is likely to result in considerably increased effectiveness of disease control [18].

In conclusion, our findings emphasize that direct contacts between badgers and cattle at pasture are very rare despite ample opportunity for interactions to occur. Future studies aiming to quantify TB risk to cattle from badgers might be best to focus on indirect contacts occurring at latrines and on direct or indirect contacts with badgers occurring away from pasture, for example in farm buildings. The observed variation in contact behaviour between badgers and cattle highlights the potential importance in understanding behavioural heterogeneity when developing strategies to control disease spread in wildlife and livestock hosts.

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DECLARATION OF INTEREST

None.

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