Supplemental Information: Badger social networks correlate with tuberculosis infection

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Supplemental Results

Numbers of animals testing positive to either Interferon Gamma Release Assay and/or Stat-Pak tests in this sample did not differ significantly between sexes (males TB+ = 13 (54%), TB- = 11 (46%); females TB+ = 8 (30%), TB- = 19 (70%); Fisher's exact test, P = 0.09) or between age classes (adult TB+ = 13 (50%), TB- = 13 (50%); sub-adult TB+ = 8 (32%), TB- = 17 (68%); Fisher's exact test, P = 0.26). TB+ and TB-badgers did not differ significantly in terms of their capture frequency (mean captures \pm SD, TB+ = 7.8 \pm 3.2; TB- = 6.5 \pm 2.9; Student's t-test, $t_{40.5}$ = 1.48, P = 0.15). TB+ and TB-badgers were similar in terms of their body condition (TB+ = 0.93 \pm 0.09, TB- = 0.89 \pm 0.09, $t_{28.4}$ =1.03, P = 0.31) and bite wound score (TB+ and TB-mode = 0, range = 0-4, Wilcoxon rank-sum test: P = 0.49).

Table S1. Degree and closeness centrality of tuberculosis test-negative (TB-) and test-positive (TB+) badgers. Degree and closeness are calculated at three different levels with different units for each: $\alpha = 0$ (based on the number of ties, i.e. value is the number of different individuals contacted), $\alpha = 0.5$ (equal weighting to tie number and strength, i.e. value is composite of tie number and duration) and $\alpha = 1$ (based on tie strength, i.e. value is duration of all contacts in seconds). Differences between TB- and TB+ individuals were tested with the UCINET node-level t-test [S11, S14] where TB test outcomes were permuted among nodes in 10,000 randomisation trials. Values are means (standard deviation) and P values represent the proportion of trials where the difference between groups was as extreme as observed. Significant values are in bold.

				1	Within-group	Among-group			
	Season	α	n	TB-	TB+	Р	TB-	TB+	Р
Degree	Summer	0	39	5.4 (2.4)	4.3 (2.3)	0.17	1.3 (0.9)	2.3 (3.0)	0.11
		0.5		1160 (734)	992 (662)	0.45	71 (106)	121 (280)	0.54
		1		282723 (239715)	242477 (194255)	0.57	7726 (14573)	9549 (24706)	0.80
	Autumn	0	44	5.7 (2.5)	4.1 (2.2)	0.04	0.8 (0.9)	0.9 (1.4)	0.91
		0.5		748 (374)	465 (350)	0.02	15 (44)	25 (49)	0.51
		1		105127 (67230)	61796 (58961)	0.03	1079 (4892)	1439 (3910)	0.77
	Winter	0	37	4.5 (1.5)	3.1 (1.5)	0.01	1.4 (1.1)	2.0 (2.4)	0.32
		0.5		943 (550)	532 (373)	0.02	46 (86)	85 (107)	0.24
		1		226236 (207380)	116974 (105573)	0.05	2526 (7250)	4634 (7328)	0.42
	Spring	0	33	4.3 (2.2)	3.1 (2.0)	0.15	1.8 (1.3)	1.1 (1.9)	0.29
		0.5		599 (437)	357 (267)	0.10	19 (32)	25 (27)	0.55
		1		110543 (121237)	68289 (89322)	0.39	349 (610)	437 (629)	0.72
Closeness	Summer	0	39	5.6 (2.3)	4.3 (2.5)	0.12	7.2 (4.9)	7.9 (6.0)	0.70
		0.5		5.6 (3.3)	5.0 (3.3)	0.60	4.4 (4.5)	4.0 (5.4)	0.82
		1		5.9 (4.3)	5.3 (3.9)	0.69	2.7 (4.7)	3.0 (6.3)	0.88
	Autumn	0	44	6.1 (2.4)	4.0 (2.5)	0.02	1.9 (1.0)	1.0 (2.1)	0.26
		0.5		6.1 (2.9)	3.7 (2.9)	0.02	1.3 (2.4)	1.0 (2.2)	0.74
		1		6.3 (3.9)	3.6 (3.3)	0.04	1.1 (3.7)	0.9 (2.9)	0.89
	Winter	0	37	4.7 (1.3)	2.8 (1.5)	<0.001	6.3 (3.9)	6.7 (5.6)	0.81
		0.5		4.5 (2.6)	2.7 (1.6)	0.05	5.2 (4.3)	6.0 (5.2)	0.64
		1		4.6 (4.3)	2.8 (1.4)	0.04	3.1 (4.8)	3.6 (3.5)	0.75
	Spring	0	33	4.3 (2.1)	2.9 (1.9)	0.09	5.6 (3.8)	4.7 (4.5)	0.59
		0.5		4.1 (3.2)	3.7 (2.2)	0.73	4.8 (3.6)	4.7 (4.7)	0.94
		1		4.2 (5.0)	4.3 (4.0)	0.93	3.5 (3.5)	3.9 (4.2)	0.77

Table S2. Flow-betweenness centrality of tuberculosis test-negative (TB-) and test-positive (TB+) badgers. Flow-betweenness is a measure of the contribution of an individual to all possible maximum flows across the population, here calculated separately within and among social groups. Differences between TB- and TB+ individuals were tested with the UCINET node-level t-test [S11, S14] where TB test outcomes were permuted among nodes in 10,000 randomisation trials. Values are means (standard deviation) and *P* values represent the proportion of trials where the difference between groups was as extreme as observed. Significant values are in bold.

	Wi	ithin-group		Among-group			
n _	TB-	TB+	Р	TB-	TB+	Р	
39	6.2 (10.2)	2.8 (6.6)	0.23	12.8 (32.7)	79.0 (146.0)	0.01	
44	8.1 (7.5)	2.6 (3.7)	0.003	3.8 (7.8)	4.3 (17.1)	0.96	
37	6.0 (7.6)	2.5 (5.9)	0.15	19.0 (38.7)	88.0 (142.0)	0.02	
33	4.7 (5.2)	3.3 (6.8)	0.54	25.2 (53.4)	35.9 (57.2)	0.61	
3	39 14 37	TB- 39 6.2 (10.2) 44 8.1 (7.5) 37 6.0 (7.6)	TB- TB+ 39 6.2 (10.2) 2.8 (6.6) 44 8.1 (7.5) 2.6 (3.7) 37 6.0 (7.6) 2.5 (5.9)	TB- TB+ P 39 6.2 (10.2) 2.8 (6.6) 0.23 44 8.1 (7.5) 2.6 (3.7) 0.003 37 6.0 (7.6) 2.5 (5.9) 0.15	TB- TB+ P TB- 39 6.2 (10.2) 2.8 (6.6) 0.23 12.8 (32.7) 44 8.1 (7.5) 2.6 (3.7) 0.003 3.8 (7.8) 37 6.0 (7.6) 2.5 (5.9) 0.15 19.0 (38.7)	TB- TB+ P TB- TB+ 39 6.2 (10.2) 2.8 (6.6) 0.23 12.8 (32.7) 79.0 (146.0) 44 8.1 (7.5) 2.6 (3.7) 0.003 3.8 (7.8) 4.3 (17.1) 37 6.0 (7.6) 2.5 (5.9) 0.15 19.0 (38.7) 88.0 (142.0)	

Table S3. Analysis of variation in degree centrality of badgers in relation to proportion of time spent at outlying setts. Degree centrality here is based on the duration of all contacts within and among groups, in seconds. Results are the output of a generalized linear mixed effects model, with season, age and sex as fixed effects and individual nested in social group as random error terms.

	Within-group				Among-group				
	Estimate	SE	t	Р	Estimate	SE	t	Р	
Time at outlying sett	-2584	525	-4.92	<0.001	130	46	2.80	0.01	
Season				<0.001				0.55	
Spring	-38691	30091	-1.29		-1355	2937	-0.46		
Summer	281668	30463	9.25		3059	3022	1.01		
Winter	71904	28029	2.57		1108	2761	0.40		
Age	86567	33176	2.61	0.01	879	2396	0.37	0.67	
Sex	36464	33522	1.09	0.26	259	2182	0.12	0.83	
Intercept	98392	45425	2.61		815	2565	0.32		

Supplemental Experimental Procedures

Study site and population

Fieldwork was conducted over a 12-month period (June 2009 – May 2010) in a highdensity badger population at Woodchester Park, Gloucestershire, UK (51°71'N, 2°30'W). The study area comprises approximately 7 km² of fragmented deciduous and coniferous woodland, agricultural grassland and smaller areas of arable and scrub. This badger population is undisturbed by culling and has been the subject of long-term ecological and epidemiological research; consequently the methods employed for their study are all well-established [e.g. S1]. Briefly, badger social group territories are mapped out on an annual basis by the deployment of marked baits at all main setts in the area and the recovery of markers in faeces at latrines [S2]. Traps are set at active setts four times a year. Captured badgers are permanently marked with a unique tattoo at first capture. At every capture they are anaesthetized, weighed, measured, reproductive condition and bite wounds are assessed (see below) and a series of samples (blood, faeces, urine, tracheal and oesophageal swabs and swabs of wounds or abscesses) are taken to determine their status with respect to infection by Mycobacterium bovis, the causative agent of TB in badgers and cattle. Assignment of individuals to a particular social group was based on capture locations with respect to territory configuration.

We calculated the proportion of adult and sub-adult badgers trapped as part of the long-term study over the period 1985-2010 to be 79% (95% confidence intervals 77–81%), by summing the number trapped (1128) and dividing this by the estimated gross population size (1431, 1394–1468), derived from the POPAN formulation [S3] of Jolly-Seber models in Program MARK version 7.1 [S4].

In 2009, 20 social groups were present in the whole study area, with a total adult and sub-adult population size, estimated as above, of 164 (134–201), of which we trapped 111 (68%, 55–83%) over the 12 month period. We sampled eight social groups living in the centre of the population and trapped 64 adult and sub-adult badgers in these groups over the year. In May and June 2009, we trapped and tagged 51 badgers (80% of the captured animals), comprising 25 sub-adults (sexually immature yearling/young-adult >1 and <2.5 years old) and 26 adults (>2.5 years), ensuring even representation of males (n = 24) and females (n = 27). Five juveniles (cubs < 1 year old) were trapped in the 8 groups in this period, but were still growing and so they could not be collared for welfare reasons. All marked badgers returning contact data were included in the network diagram (Figure 1). Forty-four badgers retained devices that collected data across two or more seasons (summer n = 39, autumn n = 44, winter n = 37, spring n = 33) before the collars either fell off or were removed 12 months after initial deployment, and these animals were included in analyses of social network and sett use.

Body condition index [S5] was calculated using the relationship between body length and weight; Condition = W / aL^n , where W is weight, L is body length, and a and n are constants. Linear regression of ln W against ln L from badgers captured during the long-term study at Woodchester Park from 1997-2009 allowed the constants a and n to be estimated separately for male and female badgers. Animals were given a score based on the number of fresh bite wounds present at the time of capture during the 2009/10 trapping season (0 = 0 wounds, 1 = 1-2 wounds, 2 = 3-4 wounds, 3 = 5+ wounds) [S6].

Infection status

The infection status of each badger was inferred from the positive or negative outcome of a combination of two diagnostic tests conducted on multiple occasions prior to and at the start of the study period: a) a badger-specific lateral flow immunoassay (BrockTB Stat-Pak; Chembio Diagnostic Systems, New York, USA) and b) an interferon-gamma release assay (IFNy). Full details of these tests and their performance are provided in [S7, S8]. For each individual, a positive result with either diagnostic test at any time was interpreted as evidence of infection with *M. bovis*. The sensitivity and specificity of the combined use of these two tests was at least 85% and 93%. In practice, all animals that tested positive to the Stat-Pak test positive also tested positive to the IFNy test. Test performance was assumed to be similar for animals irrespective of network position, thus it is unlikely that the distribution of any of a small number of false negatives or positives could have biased any trends detected, and so we did not take into account diagnostic uncertainty in our network analyses. There was, however, potential for the frequency of capture to influence the determination of infection status, since animals that had been captured and tested more frequently have a greater probability of giving a positive result. Consequently, the numbers of capture events for TB+ and TB- animals were compared to ensure that both were sampled with comparable intensity.

Contact rates

Proximity logging devices (Sirtrack, Havelock North, New Zealand) were mounted on leather collars. The loggers operate by transmitting a unique Ultra-High Frequency (UHF) code whilst simultaneously 'listening' for the codes of other loggers [S9].

Proximity loggers yield two types of information: 1) the number of discrete contacts

between pairs of individuals (contact frequency) and 2) the total duration of interactions between them (contact duration). Loggers were individually set to begin recording a contact when two or more animals came within 0.64 ± 0.04 m of one another (UHF settings range 34 - 48), and to log the time and duration of the contact to the tag's memory after the animals had been out of this range for 30 seconds or more. This short-range detection distance was chosen to record direct contacts such as mating, fighting and grooming, as well as to be within the likely aerosol transmission distance for *M. bovis* [S10].

Contact data were downloaded whenever collared badgers were recaptured. Prior to analysis, all contacts recorded during trapping operations and 12 hours after the release of the animal were removed. Data were adjusted by amalgamating contacts recorded within 1.5 minutes of each other if they involved the same pair of loggers and then removing any remaining interactions that lasted for 1 second to construct a more realistic representation of what had most likely occurred in the field [S9].

Network measures and analytical methods

Symmetrical association matrices from the raw data values for contact duration and frequency were constructed, and were found to be significantly and highly correlated with one another in all seasons (Pearson's product-moment correlation; all r > 0.93, all P < 0.001). Within-group and among-group population-level networks were analysed separately and in each of the four seasons (summer: June – August 2009; autumn: September – November 2009; winter: December 2009 – February 2010; spring: March – May 2010) to reflect these two different levels of contacts in a population structured by territorial social groupings.

Degree centrality and closeness centrality [S11] were calculated using the R package 'tnet' [S12] which allowed the relative importance given to tie number (i.e. number of individuals to which an individual is connected) and tie weight (i.e. the amount of time spent interacting with other individuals) to be varied [S13]. Flow-betweenness was calculated using UCINET [SS14]. Flow-betweenness [S11, S15] was selected as a network measure because it represents the influence an individual node holds in overall flow across the network [S15]. Flow-betweenness determines flow on the basis of all independent paths connecting pairs of nodes in the network [S15]. We take this measure as an index of potential transmission of infection and since there is no reason to assume infection is restricted to the shortest paths in the network, this measure may be a more realistic depiction of an infection network than "standard/Freeman" betweenness [S16]. Flow-betweenness has also previously been found to be correlated with infectivity, and was highly consistent with long-term infectivity [S17].

Because our sampling frame is spatially restricted, there is an edge, as in any study of finite spatial extent and in all social network analyses. Individuals living in groups at the edges of the study area will have higher actual among-group network metrics than we have recorded, due to interactions with animals from unsampled groups living outside the edge. However, numerous interfaces among groups were represented within this sample and so any putative mitigation of an edge effect, e.g. by increasing the sampling area, might be expected to change network measures in proportion to the observed trends, i.e. animals with high measures of among-group interactions stay high and animals with low measures stay low, and would therefore be expected to maintain effect size but increase statistical power.

Social network measures were compared between TB+ and TB- badgers using node-level t-tests in UCINET [S11, S14], which is the standard software for social network analysis. Regular statistical tests cannot be used on network data, because behavioural measures of individual nodes in the network are not independent of each other. UCINET accounts for the non-independence of data points in the network by using permutation trials to generate a sampling distribution of the difference between two means. For each of 10,000 trials, the scores for the network measure of interest are randomly assigned to TB+ or TB- groups (proportional to the number of each). The P value is effectively an "exact" test and is the proportion of permutation trials for which the difference between the means of TB+ and TB- groups is as extreme as the initial observation [S11, S14]. A visual representation of the whole measured network was produced using NetDraw [S18].

Sett use patterns

We investigated the influence of additional behaviours associated with badger contact patterns that may help focus management interventions. Using conventional VHF telemetry we located badgers in resting locations in main setts (large setts shared with other members of the group, usually approximately central to the social group territory) and outlying setts (smaller setts, often in the periphery of the social group territory). A fuller account of sett classification and behavioural methods is provided in [S19]. Generalised linear mixed effects models (with individual nested within social group, with both as random error terms) were used to test whether an individual's degree centrality could be predicted by the proportion of time spent at outlying setts, whilst controlling for season, age and sex effects. These analyses

were carried out using the *lmer* procedure in package *lme4* in R v. 2.11.1 (R Development Core Team 2010).

Supplemental References

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