PROCEEDINGS OF THE ROYAL SOCIETY

BIOLOGICAL SCIENCES

Who infects whom? Social networks and tuberculosis transmission in wild meerkats

Julian A. Drewe

References	This article cites 43 articles, 9 of which can be accessed free http://rspb.royalsocietypublishing.org/content/277/1681/633.full.html#ref-list-1
Subject collections	Articles on similar topics can be found in the following collections behaviour (981 articles) ecology (1106 articles) health and disease and epidemiology (179 articles)
Email alerting service	Receive free email alerts when new articles cite this article - sign up in the box at the top right-hand corner of the article or click **here**

To subscribe to *Proc. R. Soc. B* go to: **http://rspb.royalsocietypublishing.org/subscriptions**

Who infects whom? Social networks and tuberculosis transmission in wild meerkats

Julian A. Drewe[1,2,*,†]

[1]*Department of Veterinary Medicine, University of Cambridge, Madingley Road, Cambridge CB3 0ES, UK*
[2]*Department of Zoology, University of Cambridge, Downing Street, Cambridge CB2 3EJ, UK*

Transmission of infectious diseases is strongly influenced by who contacts whom. Despite the global distribution of tuberculosis (TB) in free-living wild mammal populations, little is known of the mechanisms of social transmission of *Mycobacterium bovis* between individuals. Here, I use a network approach to examine for correlations between five distinct types of intra- and intergroup social interaction and changes in TB status of 110 wild meerkats (*Suricata suricatta*) in five social groups over two years. Contrary to predictions, the most socially interactive animals were not at highest risk of acquiring infection, indicating that in addition to contact frequency, the type and direction of interactions must be considered when quantifying disease risk. Within social groups, meerkats that groomed others most were more likely to become infected than individuals who received high levels of grooming. Conversely, receiving, but not initiating, aggression was associated with *M. bovis* infection. Incidence of intergroup roving by male meerkats was correlated with the rovers themselves subsequently testing TB-positive, suggesting a possible route for transmission of infection between social groups. Exposure time was less important than these social interactions in influencing TB risk. This study represents a novel application of social network analysis using empirical data to elucidate the role of specific interactions in the transmission of an infectious disease in a free-living wild animal population.

Keywords: epidemiology; meerkat; *Mycobacterium bovis*; social network analysis; *Suricata suricatta*; tuberculosis

1. INTRODUCTION

The social structure of animal populations significantly influences the transmission dynamics and persistence of infectious diseases (Altizer *et al.* 2003; Perkins *et al.* 2008; Woodroffe *et al.* 2009). Knowledge of host interaction patterns is critical if we are to understand infectious disease transmission within a population (Cross *et al.* 2005). Despite this, few studies have examined the role of specific social interactions in the transmission of infectious agents within free-ranging populations of wild animals. Consequently, disease transmission models rarely go beyond a theoretical exploration of the influence of host ecology on disease transmission (e.g. Keeling 2005; Lloyd-Smith *et al.* 2005). This is particularly true for wildlife species owing to inherent difficulties with collecting empirical data. The role of social interaction in infectious disease transmission is an area which is crucial to understand if effective management strategies for diseases such as tuberculosis (TB) in wild animal populations are to be developed (Cross *et al.* 2009). In particular, for social animals, we need to know which interactions are associated with the transmission of infection within and between groups. We may then use this information to predict which individuals are more likely to transmit or receive, and which might (by their behaviour) be more susceptible to, or protected from, infection.

The effects of network structure on disease dynamics is an important and developing area of research (Corner *et al.* 2003*a*; Keeling 2005; Read *et al.* 2008; Perkins *et al.* 2009). For social animals, an individual's position in a group may influence risk of infection. In an infection–transmission study of TB caused by *Mycobacterium bovis* in captive brushtail possums (*Trichosurus vulpecula*), individuals who became infected were found to have greater closeness and flow-betweenness scores— that is, they were better connected to other possums in a network of den-sharing events—than those that remained free of infection (Corner *et al.* 2003*a*). Standard statistical analyses gave similar results but the network-specific measures were more precise and had the added benefit that they could be compared across time and between groups (Corner *et al.* 2003*a*). However, while different types of social interaction often produce different structures of contact network (Drewe *et al.* 2009*c*; Madden *et al.* 2009), with direct implications for disease transmission, this study was limited to the investigation of only one interaction type (den-sharing) and did not address intergroup transmission (Corner *et al.* 2003*a*). Further, the contact structure of a free-living population of wild possums is likely to be different from that of a captive population.

Quantification of the rates of social interactions between animals is the first step to determining their role in disease transmission. Recently, proximity loggers revealed all Tasmanian devils (*Sarcophilus harrisii*) within a study population to be interacting within a single contact network with potentially catastrophic consequences, since this would permit devil facial tumour disease

*jdrewe@rvc.ac.uk
[†]Present address: Royal Veterinary College, University of London, Hawkshead Lane, Hatfield, Herts AL9 7TA, UK.

(an infectious cancer threatening the species with extinction) to spread throughout the population from any single infected individual (Hamede *et al.* 2009). For group-living species such as European badgers (*Meles meles*), variation in the rates of intra- and intergroup associations between individuals is likely to profoundly affect the maintenance and transmission of infectious diseases such as TB (Böhm *et al.* 2008). Both individual- and group-level movements of badgers have been found to be positively correlated with the likelihood of becoming infected with *M. bovis* (Vicente *et al.* 2007). However, limitations in data collection technique—such as a reliance on the trapping of badgers to quantify intergroup movement rates—meant that the roles of specific behaviours in the transmission of *M. bovis* were not quantified in this study.

Meerkats (*Suricata suricatta*) are small (<1 kg) mongooses from the arid regions of southern Africa. They live in groups of up to 40 individuals consisting of a dominant female and male and a variable number of helpers of both sexes who aid in rearing the young (Clutton-Brock *et al.* 2001). Interactions within meerkat groups may be antagonistic, such as aggressive dominance assertions (Kutsukake & Clutton-Brock 2006*a*) and eviction of subordinate females (Clutton-Brock *et al.* 1998*a*), or placatory, such as grooming (Kutsukake & Clutton-Brock 2006*b*; Madden & Clutton-Brock 2009). Interactions between different groups consist principally of temporary intergroup forays by roving males (Young *et al.* 2007) and aggressive encounters between entire groups (Drewe *et al.* 2009*c*). A population of wild meerkats living in southern Kalahari has been the focus of detailed behavioural ecology studies since 1993 (Clutton-Brock *et al.* 1998*b*). Tuberculosis owing to infection with a member of the animal-adapted lineage of the *M. tuberculosis* complex (probably *M. bovis*) is endemic within this study population (Drewe 2009). Habituation of these meerkats offers a unique opportunity to study the role of specific social interactions in the transmission of TB in a wild animal population. The results are likely to aid our understanding of TB transmission dynamics in other social mammal species, such as badgers in the UK (Delahay *et al.* 2000) and possums in New Zealand (Corner *et al.* 2003*b*). I predicted that individuals engaging in the greatest amounts of social interaction would be most at risk of contracting TB.

2. MATERIAL AND METHODS

(a) *Data collection*
Data and samples were collected at the Kalahari Meerkat Project in the Northern Cape, South Africa (26°58′ S, 21°49′ E). Further details of the study site and population are given by Clutton-Brock *et al.* (1998*b*). Detailed ad libitum observations of up to 300 individually identified meerkats in 14 social groups were made over 24 months from January 2006 to December 2007. Each group was visited on at least 4 days each week, with observation periods lasting for at least 3 h in the morning after the meerkats emerged from their burrows and for at least 1 h before they re-entered their burrow in the evening. To account for a slightly unequal number of visits to each group, data were standardized by multiplying with a correction factor (the number of half-days in the study period divided by actual number of half-day visits made to the group) to ensure that comparisons between individuals and groups were based on similar

amounts of observation time. I investigated associations between the following interactions and changes in *M. bovis* infection status of individuals: grooming (both giving and receiving), aggression (both giving and receiving), eviction of subordinate females, extra-territorial roving of males and aggressive intergroup encounters. In all cases, the identities of the initiator(s) and receiver(s) were recorded. The number of meerkats included in each of the analyses varies because it was not possible to sample every meerkat at all time points.

(b) *Definitions of social interactions*
Grooming—when one individual groomed another, typically in the morning or evening at their communal burrow (Kutsukake & Clutton-Brock 2006*b*). Subordinate individuals may groom more dominant individuals to avoid harassment (Kutsukake & Clutton-Brock 2006*b*) or as a facultative response to antagonism (Madden & Clutton-Brock 2009). When three or more animals were grooming together in a huddle, interactions were recorded as dyads with one record per dyad in the same huddle. A separate grooming bout was considered to have started if the animals resumed grooming after a pause (during which there was no grooming at all) of more than 1 min. Grooming interactions between 94 meerkats (54 males and 40 females) in four social groups were recorded, a total of 11 360 interactions (table 1).

Intragroup aggression—recorded if two meerkats competed for dominance or if one meerkat asserted its dominance over another (see also Kutsukake & Clutton-Brock 2006*a*). Interactions included biting, hitting, slamming, wrestling and chin-marking (Kutsukake & Clutton-Brock 2008). Aggressive interactions between 94 meerkats (54 males and 40 females) in four social groups were recorded, a total of 7374 interactions (table 1).

Eviction of subordinate females—repeated chasing and physical attacking of the subordinates by the dominant females (and sometimes other group members of either sex). Dominant females in the latter stages of pregnancy often forcibly evict subordinate females, as the culmination of escalating aggression over the course of several days (Clutton-Brock *et al.* 1998*b*, 2006). Evicted females may live on the group periphery for several days before often being accepted back into the group after the dominant female has given birth (Clutton-Brock *et al.* 1998*a*). Eviction of 46 subordinate female meerkats from five social groups was recorded, a total of 239 eviction events (table 2).

Intergroup movements of roving males—when a male meerkat left its original social group, either singly or as part of a coalition of males, and actively sought out and approached another group of meerkats in a non-aggressive manner (Doolan & Macdonald 1996). This usually occurred as males sought breeding opportunities in other groups (Young *et al.* 2005). Rovers were only recorded if they subsequently returned to their original group, which usually occurred on the same day. The intergroup movements of 64 male meerkats from five social groups visiting up to nine other groups were recorded, a total of 2054 interactions (table 3).

Intergroup encounters—when two or more social groups met and interacted in an aggressive manner. Such encounters are frequently very aggressive and may include chasing, fighting and excavation of burrows to dig out meerkats from another group (Drewe *et al.* 2009*c*). The intergroup encounters between five social groups (96 meerkats, 50 males and 46 females) with up to nine other groups were recorded, a total of 604 intergroup interactions (table 4).

Table 1. Associations between meerkat grooming and aggression networks and *M. bovis* infection of initiators (outdegree), receivers (indegree) and individuals acting as connections between others in the network (flow-betweenness). Regression coefficients (r) and associated probabilities (p) based on 30 000 permutations of interactions between 94 meerkats in four social groups are shown. Italic values indicate significant relationships after Bonferroni correction ($p < 0.002$).

time point	date	n		grooming outdegree	indegree	flow-betweenness	n		aggression outdegree	indegree	flow-betweenness
2006											
t1	January–March	1438	r	*0.49*	*0.37*	*0.34*	1688	r	*0.36*	*0.40*	0.32
			p	*<0.001*	*0.001*	*0.002*		p	*0.001*	*<0.001*	0.003
t2	April–June	869	r	0.08	−0.03	0.13	1201	r	−0.03	0.07	−0.04
			p	0.18	0.83	0.11		p	0.62	0.14	0.94
t3	July–September	2227	r	0.21	−0.02	0.14	1120	r	0.05	0.15	−0.03
			p	0.03	0.88	0.12		p	0.77	0.14	0.83
t4	October–December	852	r	*0.37*	0.10	0.16	948	r	0.07	*0.50*	0.10
			p	*0.001*	0.34	0.09		p	0.34	*<0.001*	0.33
2007											
t5	January–March	1085	r	0.20	0.19	−0.01	823	r	*0.42*	0.08	*0.43*
			p	0.05	0.06	0.94		p	*0.002*	0.50	*0.001*
t6	April–June	1846	r	0.26	0.08	−0.01	438	r	−0.04	0.07	−0.03
			p	0.03	0.51	0.99		p	0.71	0.53	0.82
t7	July–September	2280	r	*0.35*	0.24	0.28	698	r	0.06	0.22	−0.08
			p	*0.002*	0.02	0.01		p	0.62	0.04	0.49
t8	October–December	763	r	0	0.02	0.02	458	r	−0.01	0.12	0.21
			p	1.00	0.90	0.93		p	0.94	0.14	0.06
total	2006–2007	11 360	r	*0.32*	0.27	0.14	7374	r	0.19	*0.36*	*0.30*
			p	*0.001*	0.005	0.18		p	0.08	*<0.001*	*0.001*

Table 2. Associations between aggressive evictions of subordinate females from meerkat groups (outdegree) and *M. bovis* infection of the evicted individuals. Regression coefficients (r) and associated probabilities (p) based on 30 000 permutations of 46 female meerkat evictions from five social groups are shown. No relationships are significant after Bonferroni correction ($p < 0.006$).

time point	date	number of evictions		outdegree
2006				
t1	January–March	51	r	0.19
			p	0.22
t2	April–June	19	r	0
			p	1.00
t3	July–September	54	r	−0.12
			p	0.80
t4	October–December	38	r	−0.14
			p	0.58
2007				
t5	January–March	14	r	0.12
			p	0.32
t6	April–June	14	r	−0.09
			p	0.97
t7	July–September	26	r	0.05
			p	0.89
t8	October–December	23	r	0.35
			p	0.04
total	2006–2007	239	r	−0.01
			p	0.96

(c) *Mycobacterium bovis* infection status of individual meerkats

I sampled 110 meerkats in five social groups, each up to eight times, every three months between January 2006 and December 2007 (a total of 362 samples). Median age of meerkats at first sampling was 10 months (range, 3–89 months). Details of the sampling procedure are given elsewhere (Drewe *et al.* 2009*a*). Briefly, meerkats were caught by hand and anaesthetized with isoflurane (Isofor; Safe Line Pharmaceuticals, Johannesburg, South Africa) administered by face mask. Blood was collected and subjected to two serological tests to detect presence of mycobacterial antibodies, and a tracheal wash was undertaken for mycobacterial culture (for test details see Drewe *et al.* 2009*a*). Test results for serology and culture were interpreted in parallel, meaning tests at each time point were run concurrently with a positive diagnosis requiring that only one test result be positive. This was done to maximize diagnostic sensitivity (the ability of the tests to correctly identify infected animals as test-positive) at 89 per cent (95% CI: 75–97%), while diagnostic specificity (the ability of the tests to correctly identify non-infected animals as test-negative) was 72 per cent (95% CI: 48–82%; figures calculated from data in Drewe *et al.* 2009*a*). Each meerkat was classified as test-positive for TB from the first time point at which a positive serological result was obtained or *M. bovis* was cultured from a tracheal wash sample; otherwise it was considered to be test-negative for TB.

(d) *Social network measures*

I calculated three measures of social network centralization for each meerkat: *outdegree*, an indication of the proportion of interactions *initiated* by a focal animal; *indegree*, an indication of the proportion of interactions *received* by a focal animal (Wasserman & Faust 1994); and *flow-betweenness*, an indication of the prominence or 'importance' of each meerkat in the network calculated from the number of direct and indirect connections passing through it as a

Table 3. Associations between intergroup movements of roving male meerkats and changes in *M. bovis* infection status in those males and in members of groups being visited. Outdegree data ('rovers leaving') refers to temporary departures of 64 male meerkats from five social groups. Indegree data ('rovers visiting') refers to visits to 96 meerkats in five social groups by rovers from up to nine other social groups. Regression coefficients (r) and associated probabilities (p) based on 30 000 permutations are shown. Italic values indicate significant relationships after Bonferroni correction ($p < 0.006$).

		rovers leaving			rovers visiting		
time point	date	n		outdegree	n		indegree
2006							
t1	January–March	265	r	0.03	36	r	−0.07
			p	0.83		p	0.47
t2	April–June	190	r	0	48	r	0
			p	1.00		p	1.00
t3	July–September	292	r	0.05	151	r	0
			p	0.75		p	0.98
t4	October–December	319	r	*0.58*	85	r	0.04
			p	*<0.001*		p	0.68
2007							
t5	January–March	162	r	*0.37*	33	r	0.11
			p	*0.006*		p	0.27
t6	April–June	74	r	0	31	r	−0.01
			p	1.00		p	0.91
t7	July–September	79	r	0.38	49	r	−0.09
			p	0.01		p	0.59
t8	October–December	173	r	0.13	67	r	0.13
			p	0.05		p	0.50
total	2006–2007	1554	r	*0.42*	500	r	−0.07
			p	*0.001*		p	0.48

Table 4. Associations between networks of aggressive intergroup interactions and risk of *M. bovis* infection in meerkats within those groups. Regression coefficients (r) and associated probabilities (p) based on 30 000 permutations of intergroup interactions (degree) between five focal meerkat groups (96 meerkats) and up to 13 other social groups are shown. No relationships are significant after Bonferroni correction ($p < 0.006$).

time point	date	number of interactions		degree
2006				
t1	January–March	69	r	−0.09
			p	0.38
t2	April–June	122	r	0
			p	1.00
t3	July–September	70	r	0.03
			p	0.86
t4	October–December	107	r	0.06
			p	0.57
2007				
t5	January–March	132	r	0.23
			p	0.02
t6	April–June	49	r	−0.01
			p	0.94
t7	July–September	24	r	0.19
			p	0.03
t8	October–December	31	r	0.18
			p	0.07
total	2006–2007	604	r	0.16
			p	0.11

proportion of the total flow in the network (Hanneman & Riddle 2005). If social interactions are interpreted as representing the potential flow of disease within a network, then flow-betweenness is a measure of the number of paths that pass through a focal meerkat along the shortest path between all other meerkats (Freeman *et al*. 1991). It has been suggested that an individual lying on the shortest path regulates the flow of information (e.g. disease) between two indirectly linked individuals (Borgatti 2005). The higher the flow-betweenness score, the more influential an individual is as an intermediary for contact between others. If an individual with high flow-betweenness centrality is removed from the network, the speed and certainty of transmission of infectious disease from a random individual within the network to another is more affected than if an individual with a low score is removed (Borgatti 1995).

All networks were constructed using weighted data (that is, I considered relative amounts of each interaction rather than simply recording the presence or absence of an interaction). For infections such as *M. bovis* that require close contact for transmission to occur, both the regularity of encounters and the weight of interactions are important (Read *et al*. 2008). For each interaction type, a single network containing all meerkats was constructed and individual network measures for each meerkat were calculated. An exception was aggressive intergroup encounter degree scores, which were calculated on a group-level basis and the same score allocated to all members of that group present at the time point in question.

(e) Data analysis
I analysed data in blocks of three months (i.e. eight time points within the 24-month study period) for three reasons. First, detailed network analysis of the stability of intra- and intergroup social interactions over a variety of time intervals revealed social networks constructed from data collected over three months to be representative of both shorter time

intervals (down to one week for intragroup interactions) and longer time intervals (up to 24 months for intergroup interactions; Drewe *et al.* 2009c). Second, since it is crucial to match data collection for transmission networks to the dynamics of the particular pathogen being studied (Perkins *et al.* 2009), networks constructed using three months of interactions data are likely to be biologically meaningful given the chronic nature of TB. I made the assumption that a meerkat becoming infected during one three-month time period was unlikely to go on to infect others during the same time period owing to the apparently long incubation period of TB in meerkats (Drewe 2009) but acknowledge that this may be affected by the route of transmission. Third, the inter-sampling interval for TB testing of meerkats was approximately three months and thus relationships between social interactiveness and change in infection status could be examined together over the same time period.

A logistic regression was used to examine for associations between sex, age and dominance status, and the first TB test result of each meerkat to test for bias owing to left censorship of data. This was necessary because meerkats entered the study after birth and the most likely individuals to become infected may have already done so prior to the study. Since not all meerkats were sampled at every time point, a logistic regression was used to examine for an association between monitoring time and TB test outcome for each animal, in case exposure time was a confounding variable in the incidence of testing TB-positive. These analyses were carried out in SPSS v. 15 with sex and dominance status as binary variables, and age (in months) and monitoring time (in days) as continuous variables.

Behavioural correlates of the incidence of testing TB positive were analysed using social network analysis in UCINET (Borgatti *et al.* 2002). Individual *M. bovis* infection incidence status was recorded as a dichotomous variable (0 = negative, i.e. all serologic and culture results negative; 1 = incident case of infection, i.e. the first time point at which serology and/or culture results were positive for that animal). Infection status was used as the dependent variable, with outdegree, indegree and flow-betweenness centrality measures as individual-level explanatory variables. I tested for associations between each meerkat's social network scores and change in *M. bovis* infection status during each three-month period using node-level OLS regression tests (Hanneman & Riddle 2005). This analysis accounts for autocorrelation between data points in the network. The algorithm proceeds by first determining the slope coefficients for a regression. It then recalculates these statistics over a large number (here, 30 000) of repetitions in which covariates are randomly redistributed among nodes (meerkats), while keeping the topology of the network—and any interdependencies therein—fully intact. The p-value for each statistic is the proportion of permutations that yielded a statistic as extreme as the one initially produced. To account for multiple testing of each interaction over nine time periods, I applied a Bonferroni correction and considered relationships significant where $p < 0.006$ for tests of a single network measure, and $p < 0.002$ for tests of three network measures.

3. RESULTS

Tuberculosis was detected in the meerkat population throughout the entire study period, with the incidence of new cases testing positive ranging from 4 per cent (3/69 meerkats; time point 5) to 21 per cent (10/48 meerkats; time point 6) (figure 1). Although 22 per cent
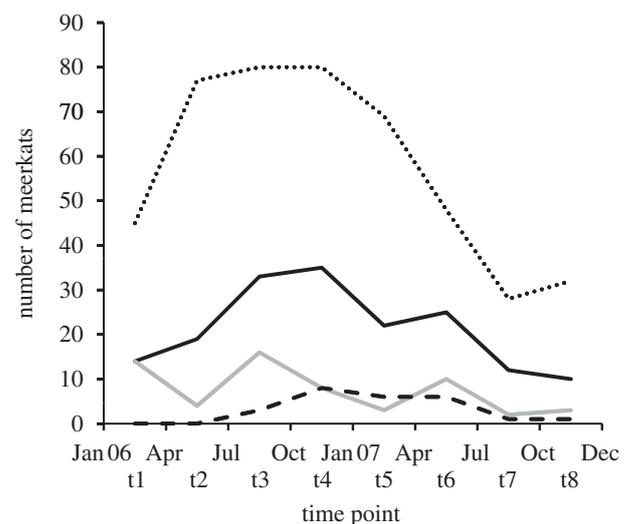
Figure 1. Tuberculosis (TB) dynamics over the study period. Meerkats were sampled at eight time points (t1–t8) throughout 2006 and 2007. The number of meerkats sampled at each time point varied owing to births and deaths. Prevalence refers to the total number of meerkats testing positive at each time point; incidence refers to new cases testing positive since the previous time point. Only deaths attributable to TB (confirmed by mycobacterial culture) are shown. Dotted line, sampled; black line, prevalence; grey line, incidence; dashed line, deaths.

(24/110) of meerkats tested positive on their first TB test, no relationships were found between the result of an individual's first TB test and its sex (odds ratio (OR) = 0.64, $p = 0.35$), age (OR = 1.02, $p = 0.29$) or dominance status (OR = 1.17, $p = 0.88$). No relationship was found between exposure time and TB test outcome (TB-negative animals: mean (range) exposure time = 380 (37–801) days; TB-positive animals = 304 (45–769) days; OR = 1, $p = 0.13$).

(a) *Is grooming between meerkats associated with becoming TB-positive by either the groomer or the groomee, or both?*
Grooming was associated with the groomer testing positive for TB at three out of the eight time points studied, and this correlation remained when all eight time points were analysed together ($p = 0.001$, table 1: grooming outdegree). Thus, meerkats that frequently groomed others were more likely to be infected than those that groomed others infrequently or not at all (figures 2a and 3a). Comparative degree distributions for meerkats of different TB test statuses are shown in figure 4a. Being groomed, however, was not generally associated with testing positive for *M. bovis* infection, although these were correlated at one time point (table 1; grooming indegree). Meerkats with high flow-betweenness scores, that is, individuals acting as links between two or more others not directly linked, generally did not show an increased risk of being infected with *M. bovis* (table 1; grooming flow-betweenness).

(b) *Is aggression between meerkats within a social group associated with acquisition of* Mycobacterium bovis *by either the aggressor or the receiver, or both?*
Aggression within meerkat social groups showed an opposite, although less clear, relationship with *M. bovis*
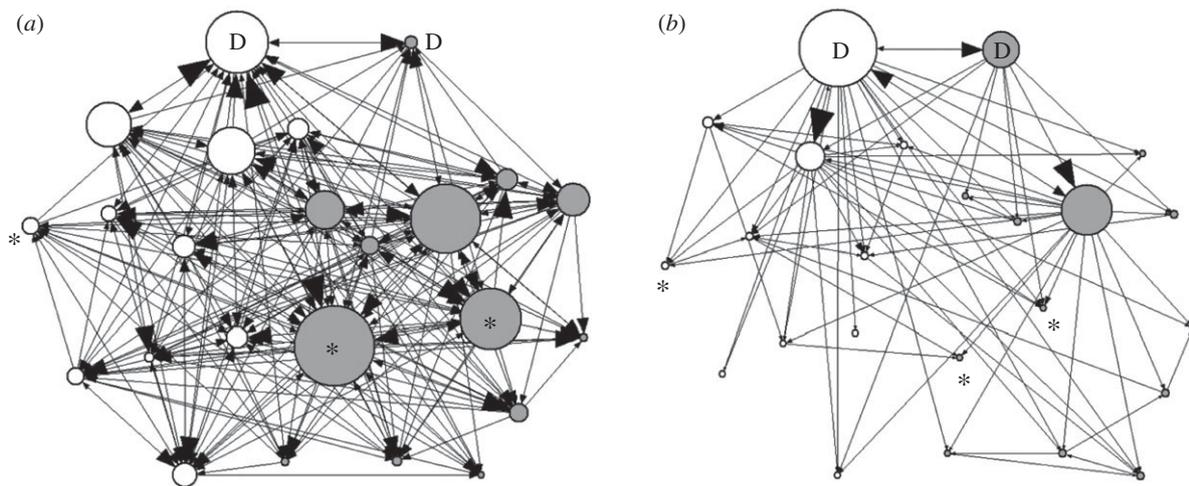
Figure 2. Social networks and TB transmission in a meerkat group. Comparative networks of (*a*) grooming and (*b*) aggressive interactions over a three-month period are shown. Each node (circle) represents a meerkat; node size is proportional to out-degree centrality (an indication of how much interaction each individual initiated). Arrowhead size is proportional to frequency of interactions, thus the sum of arrowheads around each node gives an indication of indegree centrality (the relative amount of interaction received by that meerkat). Asterisks indicate the three individuals that became TB test-positive during the time period for which the interaction data are shown. Meerkats are arranged in descending order of age from top to bottom of each diagram. White nodes, females; grey nodes, males; D, dominant individuals.
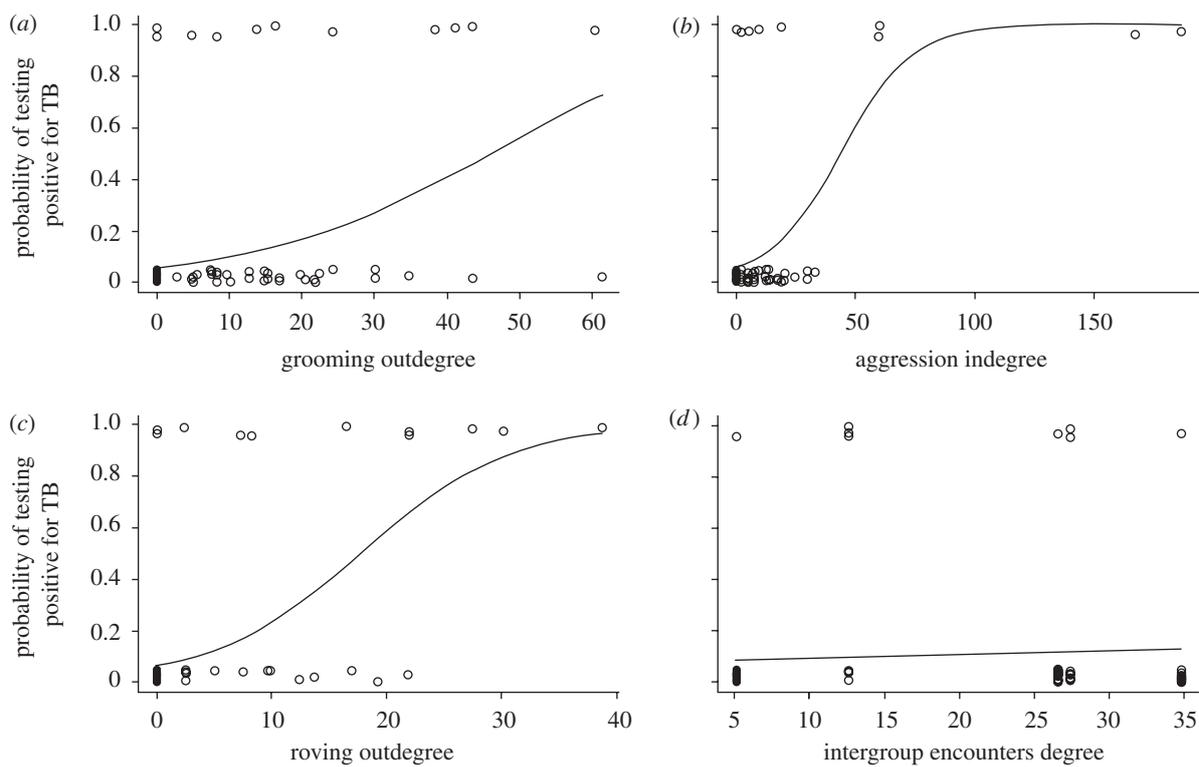
Figure 3. Fitted logistic regressions of probability of individual meerkats testing positive for TB as a function of (*a*) grooming outdegree ($n = 94$, $r = 0.37$, $p = 0.001$); (*b*) aggression indegree ($n = 94$, $r = 0.50$, $p < 0.001$); (*c*) roving male outdegree ($n = 64$, $r = 0.58$, $p < 0.001$); (*d*) intergroup encounters degree ($n = 96$ meerkats in five groups, $r = 0.06$, $p = 0.57$). Regression coefficients and their statistical significance were assessed using network permutation tests. Data shown are from time point 4 (October–December 2006).

infection status than did grooming interactions. Meerkats that initiated aggression did not show a consistent or overall increased risk of being infected with *M. bovis* although correlations existed at two time points (table 1; aggression outdegree). Meerkats that were on the receiving end of

aggression showed a significant likelihood of being infected with *M. bovis* at two of the eight time points studied, and this correlation remained when all eight time points were analysed together ($p < 0.001$, table 1: aggression indegree; figures 2*b* and 3*b*). Comparative
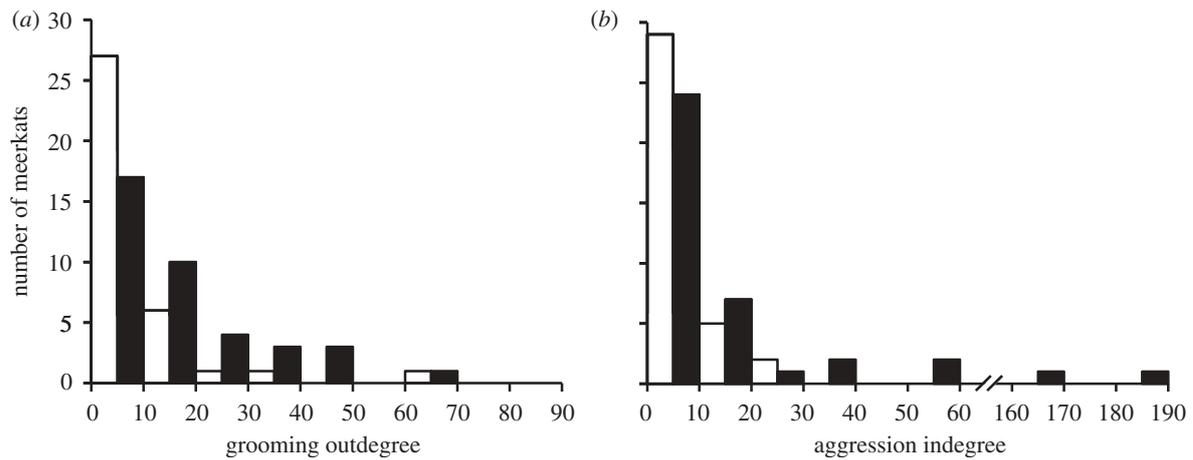
Figure 4. Degree distributions for (*a*) grooming interactions initiated and (*b*) aggressive interactions received over a three-month period (t4, October–December 2006) by meerkats testing negative (white bars) or positive (black bars) for TB at the end of this period. Both interactions were positively correlated with risk of TB infection (grooming outdegree, $r = 0.37$, $p = 0.001$; aggression indegree, $r = 0.50$, $p < 0.001$; network permutation tests with $n = 94$ meerkats in both cases).

degree distributions for meerkats of different TB test statuses are shown in figure 4*b*. Although meerkats' aggression flow-betweenness scores were not consistently associated with being infected with *M. bovis*, a significant relationship was seen when all eight time points were analysed together ($p = 0.001$, table 1; aggression flow-betweenness), suggesting that individuals acting as intermediates between others in a chain of aggressive interactions are at risk of infection with *M. bovis*.

(c) *Is temporary eviction of subordinate female meerkats from a social group associated with acquisition of* Mycobacterium bovis *by the evicted meerkat?*

No association was found between the eviction of subordinate female meerkats from a group and any change in *M. bovis* infection status of the evicted meerkats (table 2).

(d) *Is intergroup roving by male meerkats associated with a greater risk of becoming infected with* Mycobacterium bovis *by either the roving male or members of the group being visited, or both?*

Intergroup roving by male meerkats was significantly correlated with the rovers themselves being infected in two out of eight time points, and this correlation remained when all eight time points were analysed together ($p = 0.001$, table 3: outdegree; figure 3*c*). Members of either sex in groups frequently visited by rovers from elsewhere were not, however, at increased risk of being infected (table 3; indegree). This suggests that, for individual meerkats, being a rover carries more risk of TB infection than does being visited by rovers from other groups.

(e) *Is involvement in aggressive intergroup encounters associated with tuberculosis risk?*

No association was found between involvement in aggressive intergroup encounters and any change in *M. bovis* infection status of group members (table 4; figure 3*d*).

4. DISCUSSION

Exposure time was less important than involvement in specific social interactions in influencing TB risk. Infection in meerkats was more frequently associated with groomers than groomees, suggesting that meerkats which groom others are at higher risk of infection than are those that receive grooming. An uninfected meerkat that grooms an infected individual is likely to be at risk of infection by at least three routes: from inhaling infectious aerosols, from bite wounding (injection of infection) and by ingesting infectious bacteria from draining sinus tracts. While all three of these transmission routes appear to occur in meerkats (Drewe *et al.* 2009*b*), the greatest risk is likely to result from inhalation owing to the low minimum infective dose of this route: five bacilli or fewer are enough to establish pulmonary infection in cattle (Chausse 1913, cited by Phillips *et al.* 2003) compared with the oral route where several million bacilli are required to establish infection owing to the mycobactericidal effects of gastric secretions (Gaudier and Gernez-Rieux 1962, cited by Corner 2006). Thus, despite the high prevalence of discharging lymph node abscesses in infected meerkats (Drewe *et al.* 2009*b*), meerkats that ingest small amounts of infectious pus during grooming of such individuals may actually be at a low risk of establishing infection owing to the requirement for a very high oral dose (Corner 2006). Risk is likely to increase with duration of grooming but this was not measured in the present study.

Meerkats on the receiving end of aggression (those with a high aggression indegree centrality score in the preceding three months) were more likely to become infected with *M. bovis* than those that received less aggression. Although this finding was not consistent over all the eight time periods studied, a significant overall correlation was seen when all time periods were analysed together. Intragroup aggression in meerkats may result in severe bites, suggesting that direct inoculation of *M. bovis* via bite wounding may occur. Injection of infected saliva via bite wounds is thought to be an important means of TB transmission in badgers (Clifton-Hadley *et al.* 1993) and this has been linked to subsequent haematogenous

spread of *M. bovis* infection (Jenkins *et al.* 2008). Meerkat saliva may sometimes be infectious (probably because of contamination with respiratory secretions) and infected skin wounds are common in tuberculous meerkats (Drewe *et al.* 2009*b*). Based on the correlation between aggression indegree centrality, high infection rates of skin wounds (Drewe *et al.* 2009*b*) and the similarities with patterns of disease seen in badgers, it seems likely that *M. bovis* may be transmitted via bite wounding in meerkats. Meerkats that initiated aggression were overall no more likely to become infected with *M. bovis* than those that did not initiate aggression. Thus, biting others does not appear to be a significant risk factor for gaining TB by the aggressor in meerkat societies. This seems intuitive, since unless a meerkat happens to bite into an abscess on an infected individual, transmission of infection is unlikely. This goes some way to explaining why some very socially interactive dominant meerkats do not become infected. Dominant females are more likely to be groomed than to groom others (Kutsukake & Clutton-Brock 2006*b*) and are more likely to be aggressive than receive aggression (Kutsukake & Clutton-Brock 2006*a*). The present study has shown that neither of these specific behaviours (receiving grooming and initiating aggression) is related to a change in TB infection status.

While being on the receiving end of intragroup aggression was associated with becoming infected with *M. bovis*, being evicted from the group as a subordinate female was not. This is perhaps surprising, since eviction of meerkats is mediated by aggression (Stephens *et al.* 2005). However, it may be explained by the fact that during eviction events intragroup aggression originates mainly from the dominant female, who, as described above, may actually be at *low* risk of carrying infection. It is possible that the type or duration of aggression preceding eviction differs from that occurring within the group generally although no differences were observed in this study. Finally, the lack of association may be erroneous and simply related to the small sample size (239 eviction events in total over the 24-month period) and loss to follow-up of evictees who died or disappeared. More subordinate female meerkats should be sampled in future studies to clarify this.

Intergroup roving by male meerkats was associated with these individuals subsequently testing TB-positive, but not with any change in TB status of group members being visited. It is not possible to deduce from the study methodology whether it is the act of visiting other groups that carries infection risk or whether there is something else about being a rover that puts these individuals at risk of infection. Since TB status was not found to be affected by sex, age or dominance status, an individual's infection risk must be mediated by other factors. One possibility is that immunosuppressive stress hormones such as cortisol may play a role in disease susceptibility. Levels of glucocorticoid metabolites in faeces are significantly elevated in subordinate female meerkats when evicted from the safety of their group (Young *et al.* 2006). A similar increase in stress hormones in male meerkats away from their group would offer a possible explanation for the increased TB risk in roving males shown in the present study.

An important limitation of testing live animals of many species for TB is the suboptimal accuracy of diagnostic tests (Woodroffe *et al.* 1999). In particular, test sensitivity is usually low meaning early stages of infection are likely to be missed, resulting in an underestimation of the infectious proportion of the population (Chambers *et al.* 2002). To minimize the impact of false-negative test results in the present study, I used a parallel system of interpretation of three tests and considered an animal positive for TB from the time of its first positive test result onwards, resulting in an overall diagnostic sensitivity of up to 89 per cent. While this is likely to have increased the chances of correctly identifying individuals in the later stages of infection, it would not have improved detection of animals in the early stages of infection since the tests used were more likely to detect established cases of disease rather than indicate the timing of infection (Chambers *et al.* 2008). Misclassification of the TB status of some individuals means that infection may have preceded the timing of social interactions included in this study. While no changes in behaviour were observed in infected individuals until just before death (J. A. Drewe 2007, personal observation), it is possible that other meerkats may alter their behaviour towards infected individuals, perhaps targeting them with more (or less) aggression or grooming. The application of other tests that may detect early stages of infection, such as the gamma interferon test (Dalley *et al.* 2008), would be one possible solution. Logistical limitations such as the remoteness of the study site precluded the use of this test in the meerkat population.

In conclusion, transmission of *M. bovis* within meerkat groups appears to be associated with grooming and aggression, but not eviction of subordinate females. Intergroup transmission seems to be associated with roving males but not antagonistic intergroup encounters involving entire groups. These social interactions appear to be more important than the amount of exposure time in influencing the risk of testing TB-positive. Directionality of interaction appears to be important in the spread of infection, explaining why the most socially interactive individuals—the dominant male and female—are not necessarily at highest risk of infection. A similar finding was found in a study of contact networks and pathogen transmission in bumble-bees (*Bombus impatiens*) where a bee's sociality (degree centrality) did not influence its risk of infection (Otterstatter & Thomson 2007). Thus an individual's unique position in the social network (who is connected to whom) would seem to be more important than the total amount of social interaction an individual engages in, and frequent social contact (normally equated with 'high-risk' behaviour) does not necessarily increase the likelihood of infection. While not definitively proving causation, the temporal correlation between meerkats engaging in specific social interactions and testing positive for *M. bovis*, together with consideration of the pathology of this disease and the routes of infection and excretion, indicate the likely importance of grooming, biting and roving in the transmission of *M. bovis* within this meerkat population. This extends our knowledge of the mechanisms of social transmission of TB in wild mammal populations.

REFERENCES

Altizer, S. *et al.* 2003 Social organization and parasite risk in mammals: integrating theory and empirical studies. *Ann. Rev. Ecol. Evol. Syst.* **34**, 517–547. (doi:10.1146/annurev.ecolsys.34.030102.151725)

Böhm, M., Palphramand, K. L., Newton-Cross, G., Hutchings, M. R. & White, P. C. L. 2008 Dynamic interactions among badgers: implications for sociality and disease transmission. *J. Anim. Ecol.* **77**, 735–745. (doi:10.1111/j.1365-2656.2008.01377.x)

Borgatti, S. P. 1995 Centrality and AIDS. *Connections* **18**, 112–115.

Borgatti, S. P. 2005 Centrality and network flow. *Social Netw.* **27**, 55–71. (doi:10.1016/j.socnet.2004.11.008)

Borgatti, S. P., Everett, M. G. & Freeman, L. C. 2002 UCINET for Windows: software for social network analysis. Harvard, MA: Analytic Technologies. Available at http://www.analytictech.com/ucinet6/ucinet.htm.

Chambers, M. A., Pressling, W. A., Cheeseman, C. L., Clifton-Hadley, R. S. & Hewinson, R. G. 2002 Value of existing serological tests for identifying badgers that shed *Mycobacterium bovis*. *Vet. Microbiol.* **86**, 183–189.

Chambers, M. A., Crawshaw, T., Waterhouse, S., Delahay, R., Hewinson, R. G. & Lyashchenko, K. P. 2008 Validation of the BrockTB Stat-Pak assay for detection of tuberculosis in Eurasian badgers (*Meles meles*) and influence of disease severity on diagnostic accuracy. *J. Clin. Microbiol.* **46**, 1498–1500. (doi:10.1128/JCM.02117-07)

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

Clutton-Brock, T. H., Brotherton, P. N. M., Smith, R., McIlrath, G. M., Kansky, R., Gaynor, D., O'Riain, M. J. & Skinner, J. D. 1998a Infanticide and expulsion of females in a cooperative mammal. *Proc. R. Soc. Lond. B* **265**, 2291–2295. (doi:10.1098/rspb.1998.0573)

Clutton-Brock, T. H. *et al.* 1998b Costs of cooperative behaviour in suricates, *Suricata suricatta*. *Proc. R. Soc. Lond. B* **265**, 185–190. (doi:10.1098/rspb.1998.0281)

Clutton-Brock, T. H. *et al.* 2001 Cooperation, control, and concession in meerkat groups. *Science* **291**, 478–481. (doi:10.1126/science.291.5503.478)

Clutton-Brock, T. H., Hodge, S. J., Spong, G., Russell, A. F., Jordan, N. R., Bennett, N. C., Sharpe, L. L. & Manser, M. B. 2006 Intrasexual competition and sexual selection in cooperative mammals. *Nature* **444**, 1065–1068. (doi:10.1038/nature05386)

Corner, L. A. 2006 The role of wild animal populations in the epidemiology of tuberculosis in domestic animals: how to assess the risk. *Vet. Microbiol.* **112**, 303–312. (doi:10.1016/j.vetmic.2005.11.015)

Corner, L. A., Pfeiffer, D. U. & Morris, R. S. 2003a Social-network analysis of *Mycobacterium bovis* transmission among captive brushtail possums (*Trichosurus vulpecula*). *Prev. Vet. Med.* **59**, 147–167. (doi:10.1016/S0167-5877(03)00075-8)

Corner, L. A., Stevenson, M. A., Collins, D. M. & Morris, R. S. 2003b The re-emergence of *Mycobacterium bovis* infection in brushtail possums (*Trichosurus vulpecula*) after localised possum eradication. *NZ Vet. J.* **51**, 73–80.

Cross, P. C., Lloyd-Smith, J. O., Johnson, P. L. F. & Getz, W. M. 2005 Duelling timescales of host movement and disease recovery determine invasion of disease in structured populations. *Ecol. Lett.* **8**, 587–595. (doi:10.1111/j.1461-0248.2005.00760.x)

Cross, P. C., Drewe, J. A., Patrek, V., Pearce, G. P., Samuel, M. D. & Delahay, R. J. 2009 Wildlife population structure and parasite transmission: implications for disease management. In *Management of disease in wild mammals* (eds R. J. Delahay, M. R. Hutchings & G. C. Smith), pp. 9–29. Tokyo, Japan: Springer Publishing. (doi:10.1007/978-4-431-77134-0_2)

Dalley, D., Dave, D., Lesellier, S., Palmer, S., Crawshaw, T., Hewinson, G. H. & Chambers, M. A. 2008 Development and evaluation of a gamma interferon assay for tuberculosis in badgers (*Meles meles*). *Tuberculosis* **88**, 235–243. (doi:10.1016/j.tube.2007.11.001)

Delahay, R. J., Langton, S., Smith, G. C., Clifton-Hadley, R. S. & Cheeseman, C. L. 2000 The spatio-temporal distribution of *Mycobacterium bovis* (bovine tuberculosis) infection in a high-density badger population. *J. Anim. Ecol.* **69**, 428–441.

Doolan, S. P. & Macdonald, D. W. 1996 Dispersal and extra-territorial prospecting by slender-tailed meerkats (*Suricata suricatta*) in the south-western Kalahari. *J. Zool.* **240**, 59–73.

Drewe, J. A. 2009 Social networks and infectious disease transmission: epidemiology of tuberculosis in wild meerkats. PhD thesis, University of Cambridge, Cambridge, UK.

Drewe, J. A., Dean, G. S., Michel, A. L., Lyashchenko, K. P., Greenwald, R. & Pearce, G. P. 2009a Accuracy of three diagnostic tests for determining *Mycobacterium bovis* infection status in live-sampled wild meerkats (*Suricata suricatta*). *J. Vet. Diagn. Invest.* **21**, 31–39.

Drewe, J. A., Foote, A. K., Sutcliffe, R. L. & Pearce, G. P. 2009b Pathology of *Mycobacterium bovis* infection in wild meerkats (*Suricata suricatta*). *J. Comp. Pathol.* **140**, 12–24. (doi:10.1016/j.jcpa.2008.09.004)

Drewe, J. A., Madden, J. R. & Pearce, G. P. 2009c The social network structure of a wild meerkat population: 1. Inter-group interactions. *Behav. Ecol. Sociobiol.* **63**, 1295–1306. (doi:10.1007/s00265-009-0782-x)

Freeman, L. C., Borgatti, S. P. & White, D. R. 1991 Centrality in valued graphs: a measure of betweenness based on network flow. *Social Netw.* **13**, 141–154.

Hamede, R. K., Bashford, J., McCallum, H. & Jones, M. 2009 Contact networks in a wild Tasmanian devil (*Sarcophilus harrisii*) population: using social network analysis to reveal seasonal variability in social behaviour and its implications for transmission of devil facial tumour disease. *Ecol. Lett.* **12**, 1147–1157. (doi:10.1111/j.1461-0248.2009.01370.x)

Hanneman, R. A. & Riddle, M. 2005 *Introduction to social network methods*. Riverside, CA: University of California. Published in digital form at http://www.faculty.ucr.edu/~hanneman/nettext/.

Jenkins, H. E. *et al.* 2008 The prevalence, distribution and severity of detectable pathological lesions in badgers naturally infected with *Mycobacterium bovis*. *Epidemiol. Infect.* **136**, 1350–1361. (doi:10.1017/S0950268807009909)

Keeling, M. J. 2005 The implications of network structure for epidemic dynamics. *Theoret. Popul. Biol.* **67**, 1–8. (doi:10.1016/j.tpb.2004.08.002)

Kutsukake, N. & Clutton-Brock, T. H. 2006*a* Aggression and submission reflect reproductive conflict between females in cooperatively breeding meerkats *Suricata suricatta*. *Behav. Ecol. Sociobiol.* **59**, 541–548. (doi:10.1007/s00265-005-0079-7)

Kutsukake, N. & Clutton-Brock, T. H. 2006*b* Social functions of allogrooming in cooperatively breeding meerkats. *Anim. Behav.* **72**, 1059–1068. (doi:10.1016/j.anbehav.2006.02.016)

Kutsukake, N. & Clutton-Brock, T. H. 2008 Do meerkats engage in conflict management following aggression? Reconciliation, submission and avoidance. *Anim. Behav.* **75**, 1441–1453. (doi:10.1016/j.anbehav.2007.09.018)

Lloyd-Smith, J. O., Schreiber, S. J., Kopp, P. E. & Getz, W. M. 2005 Superspreading and the effect of individual variation on disease emergence. *Nature* **438**, 355–359. (doi:10.1038/nature04153)

Madden, J. R. & Clutton-Brock, T. H. 2009 Manipulating grooming by decreasing ectoparasite load causes unpredicted changes in antagonism. *Proc. R. Soc. B* **276**, 1263–1268. (doi:10.1098/rspb.2008.1661)

Madden, J. R., Drewe, J. A., Pearce, G. P. & Clutton-Brock, T. H. 2009 The social network structure of a wild meerkat population: 2. Intragroup interactions. *Behav. Ecol. Sociobiol.* **64**, 81–95. (doi:10.1007/s00265-009-0820-8)

Otterstatter, M. C. & Thomson, J. D. 2007 Contact networks and transmission of an intestinal pathogen in bumble bee (*Bombus impatiens*) colonies. *Oecologia* **154**, 411–421. (doi:10.1007/s00442-007-0834-8)

Perkins, S. E., Ferrari, M. F. & Hudson, P. J. 2008 The effect of social structure and sex-biased transmission on macroparasite infection. *Parasitology* **135**, 1561–1569. (doi:10.1017/S0031182008000449)

Perkins, S. E., Cagnacci, F., Stradiotto, A., Arnoldi, D. & Hudson, P. J. 2009 Comparison of social networks derived from ecological data: implications for inferring infectious disease dynamics. *J. Anim. Ecol.* **78**, 1015–1022. (doi:10.1111/j.1365-2656.2009.01557.x)

Phillips, C. J. C., Foster, C. R., Morris, P. A. & Teverson, R. 2003 The transmission of *Mycobacterium bovis* infection to cattle. *Res. Vet. Sci.* **74**, 1–15.

Read, J. M., Eames, K. T. D. & Edmunds, W. J. 2008 Dynamic social networks and the implications for the spread of infectious disease. *J. R. Soc. Interface* **5**, 1001–1007. (doi:10.1098/rsif.2008.0013)

Stephens, P. A., Russell, A. F., Young, A. J., Sutherland, W. J. & Clutton-Brock, T. H. 2005 Dispersal, eviction, and conflict in meerkats (*Suricata suricatta*): an evolutionarily stable strategy model. *Am. Nat.* **165**, 120–135.

Vicente, J., Delahay, R. J., Walker, N. J. & Cheeseman, C. L. 2007 Social organization and movement influence the incidence of bovine tuberculosis in an undisturbed high-density badger *Meles meles* population. *J. Anim. Ecol.* **76**, 348–360. (doi:10.1111/j.1365-2656.2006.01199.x)

Wasserman, S. & Faust, K. 1994 *Social network analysis: methods and applications*. Cambridge, UK: Cambridge University Press.

Woodroffe, R., Frost, S. D. W. & Clifton-Hadley, R. S. 1999 Attempts to control tuberculosis in cattle by removing infected badgers: constraints imposed by live test sensitivity. *J. Appl. Ecol.* **36**, 494–501.

Woodroffe, R. *et al.* 2009 Social group size affects *Mycobacterium bovis* infection in European badgers (*Meles meles*). *J. Anim. Ecol.* **78**, 818–827. (doi:10.1111/j.1365-2656.2009.01545.x)

Young, A. J., Carlson, A. A. & Clutton-Brock, T. 2005 Trade-offs between extraterritorial prospecting and helping in a cooperative mammal. *Anim. Behav.* **70**, 829–837. (doi:10.1016/j.anbehav.2005.01.019)

Young, A. J., Carlson, A. A., Monfort, S. L., Russell, A. F., Bennett, N. C. & Clutton-Brock, T. 2006 Stress and the suppression of subordinate reproduction in cooperatively breeding meerkats. *Proc. Natl Acad. Sci. USA* **103**, 12 005–12 010. (doi:10.1073/pnas.0510038103)

Young, A. J., Spong, G. & Clutton-Brock, T. 2007 Subordinate male meerkats prospect for extra-group paternity: alternative reproductive tactics in a cooperative mammal. *Proc. R. Soc. B* **274**, 1603–1609. (doi:10.1098/rspb.2007.0316)