

Influence of contact heterogeneity on TB reproduction ratio R_0 in a free-living brushtail possum *Trichosurus vulpecula* population

Thibaud PORPHYRE*, Mark STEVENSON, Ron JACKSON, Joanna MCKENZIE

EpiCentre, Institute of Veterinary, Animal, and Biomedical Sciences, Massey University, Private Bag 11222, Palmerston North 4442, New Zealand

(Received 7 September 2007; accepted 25 January 2008)

Abstract – Social network analyses were used to investigate contact patterns in a free-living possum *Trichosurus vulpecula* population and to estimate the influence of contact on R_0 for bovine tuberculosis (TB). Using data collected during a five-year capture-mark-recapture study of a free-living possum population, observed estimates of R_0 were computed and compared with R_0 computed from random networks of similar size that approximated a random mixing process. All networks displayed a heterogeneous pattern of contact with the average number of contacts per possum ranging from 20 to 26 per year. The networks consistently showed small-world and single-scale features. The mean estimates of R_0 for TB using the observed contact networks were 1.78, 1.53, 1.53, 1.51, and 1.52 times greater than the corresponding random networks ($P < 0.05$). We estimate that TB would spread if an average of between 1.94 and 1.97 infective contacts occurred per year per infected possum, which is approximately half of that expected from a random network. These results have implications for the management of TB in New Zealand where the possum is the principal wildlife reservoir host of *Mycobacterium bovis*, the causal agent of bovine TB. This study argues the relevance of refining epidemiological models used to inform disease management policy to account for contact heterogeneity.

basic reproduction ratio / *Mycobacterium bovis* / small-world / social network analysis / wildlife disease

1. INTRODUCTION

Evaluating the transmission dynamics of an infectious disease process and its ability to establish and persist in a population is essential for devising effective control strategies. A key determinant of disease spread is the basic reproduction ratio, R_0 [30, 37], which is defined as the expected (average) number of secondary infections caused by an infected host [3, 28]. Empirically, assuming a relatively large, susceptible population, a value of R_0 greater than 1 indicates that disease will spread whereas a value of less than 1 indicates that a self-sustaining epidemic is not possible and that disease will die out [3].

In wild animal populations, calculation of R_0 is challenging as it depends on knowledge of the contact structure in the population of interest, which is often unknown [3]. In their simplest form, susceptible-infected-recovered (SIR) models assume that individuals in a population are equally likely to contact and infect each other [3]. Random networks can be created to approximate features of disease transmission that are similar to stochastic SIR models [54]. In reality however, the probability of contact is not uniform across all members of a population. Non-uniformity means that the likelihood of a disease being transmitted from one individual to another will vary with the probability of an infected individual making contact with other members of the population [43]. This has important

* Corresponding author: t.porphyre@massey.ac.nz

implications for the study of disease in wild animal populations, as social groups are likely to exist which would create heterogeneity in population contact structure [20, 24, 47–49, 52, 69].

Bovine tuberculosis (TB) caused by infection with *Mycobacterium bovis* is a zoonotic disease which represents an important economic and public health concern for both developed and developing countries. Although cattle are the natural hosts for *M. bovis*, the transmission dynamics of TB involves a wide range of wildlife animals, among which the brushtail possum *Trichosurus vulpecula* in New Zealand and the European badger *Meles meles* in UK and Ireland are thought to play a role in allowing the disease to persist in the environment. Numerous models of TB transmission amongst possums and badgers have been developed to evaluate disease control strategies such as culling, vaccination, and modifying fertility. Most applications of SIR modelling to the spread of TB have been implemented using transmission rates that assume homogenous mixing (see [63] for a review). Although models developed under such assumptions have greatly assisted in understanding the transmission dynamics of bovine and wildlife TB [63], they may underestimate the rate of new infection in wildlife or livestock populations by failing to account for individual variations in contact patterns. Heterogenous mixing of populations has been incorporated in a small number of TB models [5, 61]. In these models, parameters representing heterogeneity have not been based on data derived from real populations, but have been calculated to achieve model outputs that best fits observed patterns of disease.

Social network analysis (SNA) provides a method for quantifying the contact structure within a population [65]. When studying factors influencing the spread of disease in a population the given set of relationships (or ties) that exist between individuals allows one to draw a network of potential transmission pathways. In this way, information can be gathered to identify which individuals are at greater risk of transmitting or receiving infection. Knowledge of the topography of

the network is also critical, since discerning the degree of organisation of a network not only provides useful information on the ease with which a disease can spread throughout the population [66], but also on the resilience of the network to removal of individuals [1]. Social network analysis has previously been applied to data describing the contact pattern of captive possums during denning [20]. Corner et al. [20] established that experimentally infecting highly socially interactive possums significantly increased the level of transmission of infection, compared to infecting animals at random. Although this finding is relevant in the context of captive possum populations, these observations may not be applicable to free-living possum populations.

In this paper, we describe an estimated social network structure of a free-living and uncontrolled possum population in a TB endemic area in New Zealand. Our first aim was to estimate the pattern of contact between possums and describe the topography of the estimated network. Our second aim was to quantify the relative effect of heterogeneity on the spread of TB infection in this population.

2. MATERIALS AND METHODS

2.1. Study population

The study population was comprised of all possums captured using capture-mark-recapture methodology implemented in a 22 ha study site near Castlepoint (40° 51' S, 176° 14' E) on the south-eastern coast of the North Island of New Zealand [18, 55, 56].

Trapping was conducted for three to five nights every month from 1 April 1989 to 31 August 1994 (inclusive). On each trapping night approximately 295 traps were set. In total 67 183 trapping occasions were recorded over 249 nights during the 65 month study period. A total of 740 possums (274 females, 464 males, and two with unrecorded gender) was captured and identified, representing 18 367 capture events.

Details recorded at the time of capture included age, sex, presence or absence of TB, and geographical location of trap site using a Global Positioning System (GPS). Possums were classified according to their estimated maturity stage as

follows: (i) adult (≥ 2 years of age), (ii) yearling (between 1 and 2 years), and (iii) juvenile (≤ 1 year). As spatial information for several capture locations ($n = 28$) was missing, 32 possums (13 females, 18 males, and one of unrecorded gender) were discarded from the analyses, reducing the total number of capture events by 34.

2.2. Construction of the contact network

We defined contact as occurring through the following two mechanisms: (i) direct contact within a possum's activity range, and (ii) indirect contact through sequential trap use.

Although possums are solitary and tend to avoid encounters with each other [26, 58], direct contact occurs during mating, simultaneous den sharing, agonistic encounters, and fighting [12, 22, 33, 41, 42]. Direct contact also occurs between mothers and their offspring before weaning. These behaviours have been hypothesised to be associated with the transmission of TB [17, 40]. Indirect disease-transmitting contact may occur through sequential use of dens, as a result of environmental contamination with *M. bovis* [39]. It is reasonable to assume that spatial proximity is an important factor in the disease transmission process, which infers that being spatially close increases the likelihood of disease transmission by increasing the number of both direct and indirect contacts [38].

In this study disease-transmitting contact between possums was represented on the basis of recorded capture locations. Direct contact between two possums was defined as the capture of a possum within the activity range (AR) [59] of another possum during the time period(s) during which they were both alive. The AR of each possum was defined as the area containing the 80th percentile of the kernel density surface of capture events using a quartic kernel function and arbitrarily required at least seven capture locations to be calculated. The requirement for seven capture locations was fixed in order to increase the precision of the estimated AR while maximising the number of possums for which AR was calculated (Tab. I). We used the cross-validation method to estimate the bandwidth parameter for the kernel density surface for each possum, setting the minimum bandwidth at 10 m. Although the cross validation method tends to under-smooth a point process [11], we used this approach in preference to a fixed value (as used by Ramsey and Cowan [59] and Norton et al. [55]).

Table I. Descriptive statistics of the number of capture events for possums involved in the five 12-month interval contact networks starting from April 1989 in the possum population present in the Castlepoint study site.

Period	n	Mean (SD)	Median (Q1, Q3)	Min	Max
1989–1990	269	9.0 (9.2)	5 (2, 14)	1	44
1990–1991	244	15.7 (15.0)	11 (3, 23)	1	59
1991–1992	252	12.3 (11.7)	7 (3, 19)	1	40
1992–1993	266	11.8 (10.4)	8 (3, 17)	1	36
1993–1994	279	14.3 (12.1)	11 (3, 27)	1	37

Indirect contact between two possums was defined as the capture of a possum at the same trap location within a one month lag (that is ± 1 month) of another. This was used to account for the survival time of *M. bovis* in the trap, which was considered similar to survival of the organism in a den (maximum *M. bovis* survival equal to 28 days [39]). It also accounted for the possibility of contact occurring in the area surrounding a trap.

Sets of activity range and trap contacts between possums were constructed for five 12-month periods. For the purpose of this study the start of each period was April, the beginning of the main breeding season [22]. Defining the time periods in this way allowed us to crudely account for the natural loss and gain of the population through immigration, emigration, births, and deaths and for annual variations in the frequency of contact due to mating and food availability.

Activity range and trap contact sets were combined to give a simple, undirected network, represented as a graph comprised of a set of nodes (in this case individual possums) joined by ties. In this study, contact between two animals was represented as a dichotomous response, independent of the number of times contact was actually made. Computation of the activity ranges, used to define the contact matrices were implemented using the SPLANCS [9, 62] package implemented in R version 2.3.1 (R Development Core Team, Vienna, Austria).

2.3. Statistical analyses

Each network was described using the standardised programme for empirical research of complex networks [29]. First, node degree (that is the number of different possums contacted by a

single possum) was used as a measure of centrality to determine the extent to which the network revolved around a possum [20, 65]. The frequency distribution of contact, the density (that is the number of ties of an individual as a proportion of all possible ties within the population), and the Freeman network centralisation index (expressing the number of ties in the network as a proportion of that of a perfect star network of the same size [65]) were calculated for each contact network. Second, average geodesic distance (GD; the shortest distance between any two nodes) between all pairs of nodes and the clustering coefficient (CC; measuring the degree of interconnection which may exist between neighbours of a node of interest) were calculated for each of the observed networks and compared with the GD and CC of a random network [32]. The five 12-month period random networks were Bernoulli-generated using Pajek software version 1.15 (University of Ljubljana, Ljubljana, Slovenia [8]) and were constructed so that they had similar characteristics (the same number of nodes and average degree) as the observed networks.

We were interested to know if the observed networks showed features of what is called a “scale-free” process. Networks presenting scale-free features imply the presence of individuals, called “superspreaders” [3, 70], who present a larger amount of contact than statistically expected, thereby significantly increasing the pace at which infections may spread throughout the population [7]. If κ defines the number of contacts an individual has over a 12-month period, a scale-free process is characterised by a power-law distribution of the form $P(\kappa) \sim \kappa^{-\gamma}$, where γ is a decay coefficient with $2 < \gamma \leq 3$. The scale-free properties of the networks were assessed in two ways: (i) by calculating the expected diameter of a scale-free network of similar size as $d_E = \ln \ln N$ [16], assuming $2 < \gamma \leq 3$, and (ii) by visually assessing if the degree distribution followed a power-law by plotting the cumulative distribution, $P(\kappa)$, as a function of κ [2, 44]. In a scale-free network this distribution should fall on a straight line when plotted on a log-log scale. Alternatively, two other network processes may be distinguished from the log-log plot of the degree distribution. These are “single-scale” and “broad-scale” networks [2]. A single-scale network presents a quickly decaying degree distribution in the log-log plot (that is following an exponential or Gaussian distribution), whereas a broad-scale

network has two stage features, with a degree distribution that has first a power-law regime (i.e. straight line) followed by a sharp cut-off.

Influence of either maturity or gender on the probability of having contact was assessed using a two-sided *t*-test to compare the difference in the means of the normalised degree (that is the degree divided by the maximum possible degree of the network [10, 64]). Since network data do not hold the assumption of independence between measurements, two-sided permutation-based *p*-values were computed on 10 000 replicates of a randomly selected sample of the observed data set.

All network parameters (degree, network density, Freeman network centralisation index, GD and CC) and statistical analyses were calculated using Ucinet version 6.137 for Windows (Analytic Technologies Inc., Harvard, Massachusetts, USA; [10]).

2.4. Disease modelling and basic reproduction ratio

The influence of the heterogeneity of contacts on the capacity of a pathogen to spread and persist in the possum population was examined using the basic reproduction ratio, R_0 [3]. This ratio, defined as the average number of secondary cases caused by an infected individual in a totally susceptible population [3, 37], may be estimated by:

$$R_0 = \beta \langle \kappa \rangle \langle D \rangle \times (1 + CV^2) \quad (1)$$

where β is the transmission probability per contact, $\langle \kappa \rangle$ is the average number of contacts (or degree) per year, and $\langle D \rangle$ is the median duration (in years) of infectiousness of a TB-positive possum. To account for heterogeneity in the number of contacts between individuals in the network, the coefficient of variation *CV* for node degree was introduced, which represents the ratio of the standard deviation of the degree to the average degree per 12-month period [50, 51]. It is noted that the product $\beta \langle \kappa \rangle$ represents the transmission rate used in SIR modelling.

A 95% confidence interval around the median estimate of R_0 was computed from a parametric bootstrap-generated distribution using 1 000 permutations [25, 31] assuming the observed set of contacts between possums was randomly sampled from the true contact network of the population. Negative binomial distributions were fitted using a maximum likelihood estimator over the observed distributions of: (i) contacts that occurred in

the studied population, κ , and (ii) duration of infectiousness for TB-positive possums, D (Fig. 1) [5, 13]. D was assumed to be drawn from a negative binomial distribution of values representing the time between detection of TB and death [60]. While clinical signs of disease were likely to have been promptly detected as possums were trapped and examined every month, possums may have been infectious for a period varying from two to four months before the detection of clinical signs [19, 61]. As a result this approach potentially underestimates the duration of infectiousness, with a corresponding reduction in the computed value of R_0 .

3. RESULTS

3.1. Description of network characteristics

The number of contacts between possums was relatively low in the five 12-month interval networks, with an average degree $\langle \kappa \rangle$ of 24 (range 20–26; Tab. II) from an annual population size that ranged from 244 to 279 individuals. That is, each possum contacted, on average, between 7.5% and 9.9% of the other possums in the network. Moreover, the total number of ties for each year was 5 392, 5 918, 5 582, 6 760, and 7 152, representing a network density of 15%, 20%, 18%, 19%, and 18%. Table II shows that contact networks were heterogeneous, with a degree SD ranging from 17 to 19. This is supported by a high coefficient of variation which ranged from 74% to 87% (Tab. II), consistent with a negative binomial distribution (Fig. 1). Freeman’s degree centralisation indices for each 12-month period were 27.4%, 43.9%, 30.9%, 35.2%, and 36.3% of the theoretical maximums for each period. These findings indicate that there was a substantial amount of concentration in the possum population throughout the study period and that the number of contacts between possums was unequally distributed.

For four of the five 12-month periods studied, contact among adult and yearling possums differed (Tab. III). The mean number of contacts amongst adult possums during the

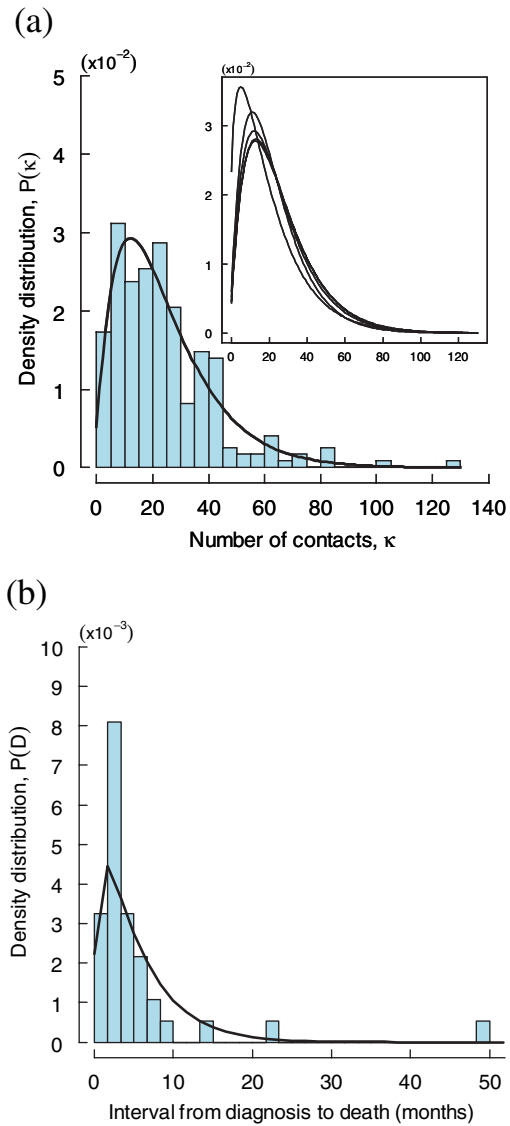


Figure 1. Density distributions of: (a) the amount of contacts κ made by the 244 possums involved in the 12-month interval contact network starting in April 1990; and (b) the interval D from bovine tuberculosis diagnosis to death of naturally infected possums living within the study site boundaries between April 1989 and August 1994. The solid line represents the negative binomial fit over the observed distributions. The inset in (a) shows the fitted density distributions of κ for the five 12-month interval contact networks.

Table II. Descriptive statistics for the five 12-month interval contact networks of free-living possums caught in the Castlepoint study site. The table provides the number of possums involved in the networks (N), the number of isolated possums (n), and the average $\langle \kappa \rangle$, standard deviation (SD), coefficient of variation (CV), range (Min, Max) of the number of contacts made by possums, and the Freeman’s network centralisation index.

Period	$\langle \kappa \rangle$	SD	Min	Max	CV	N	n	Index ^a (%)
1989–1990	20.05	17.39	0	93	0.87	269	2	27.43
1990–1991	24.25	18.46	1	130	0.76	244	0	43.88
1991–1992	22.15	17.10	1	99	0.77	252	0	30.86
1992–1993	25.41	19.23	2	118	0.76	266	0	35.20
1993–1994	25.63	18.85	2	104	0.74	279	0	36.25

^a Index: Freeman network centralisation index, expressing the degree of inequality or variance in networks as a percentage of that of a perfect star network of the same size.

second, third, fourth and fifth study years was 20 (SD = 17), 24 (SD = 17), 29 (SD = 20) and 29 (SD = 19), whereas yearlings had an average of 27 (SD = 19), 19 (SD = 17), 20 (SD = 20), and 19 (SD = 16) contacts. Differences in node degree for adults and yearlings for years 2, 3, 4, and 5 were significant at the alpha level of 0.01.

With respect to the effect of gender on the number of contacts, no trends were apparent. Although females had a significantly greater number of contacts compared with males in the third 12-month period ($\langle \kappa \rangle_F = 25$; $\langle \kappa \rangle_M = 18$; $P < 0.01$) no gender associated pattern was observed during the other years.

3.2. Topography of the network

Topography measures did not change substantially throughout the study period.

Each of the five networks had similar average geodesic distances compared with equivalent random networks (between 14% and 18% greater) but showed a considerably greater clustering coefficient (between 6.5 and 9.3 times; Tab. IV) compared with equivalent random networks. The contacts established for the studied possum population consistently displayed features of a “small-world” network; that is a network with a high level of clustering with short between-node distances [66].

Figure 2 shows the cumulative distribution of the number of contacts between possums (on the log-log scale) for each study year. The log-log plot shows that the cumulative distributions of contacts between possums did not decay with a power-law tail, and this pattern was consistent for each 12-month period. However, when the average geodesic distance of each 12-month network

Table III. Comparison of the mean (and standard deviation) of the number of contacts made by possums caught in the Castlepoint study site during the five 12-month intervals, stratified by maturity and gender. Comparisons are based on normalised contact counts using a permutation-based t -test with 10 000 iterations.

Period	Maturity			Gender		
	Yearling (SD)	Adult (SD)	P	Female (SD)	Male (SD)	P
1989–1990	19.4 (16.51)	20.7 (18.26)	0.528	20.8 (18.08)	19.1 (16.41)	0.424
1990–1991	26.6 (18.77)	19.9 (17.08)	0.006	23.1 (15.34)	25.1 (20.25)	0.416
1991–1992	18.6 (16.74)	24.2 (16.97)	0.010	24.8 (18.81)	17.6 (12.40)	0.002
1992–1993	20.1 (15.94)	28.8 (20.34)	$< 0.001^a$	22.6 (15.33)	27.0 (20.92)	0.074
1993–1994	19.2 (16.14)	29.3 (19.31)	< 0.001	24.0 (16.29)	26.6 (20.16)	0.259

^a Interpretation: on average, adult possums significantly presented $28.8 - 20.1 = 8.7$ more contacts ($P < 0.001$) than yearling possums during the period from April 1992 to March 1993 (inclusive).

Table IV. Average geodesic distance (GD_{Obs}) and clustering coefficient (CC_{Obs}) of the five 12-month interval contact networks of free-living possums caught in the Castlepoint study site. For comparison, the average geodesic distance (GD_{Rand}) and the clustering coefficient (CC_{Rand}) of random networks of similar size (N) are shown. The table provides the mean number degree κ_{ER} of the random network and the expected diameter d_E if the network is scale-free with $2 \leq \gamma \leq 3$. The expected diameters are computed using $d_E = \ln \ln N$ [16].

Period	N	Ties	κ_{ER} (SD)	d_E	Observed network		Random network	
					CC_{Obs}	GD_{Obs}	CC_{Rand}	GD_{Rand}
1989–1990	269	5 392	20.1 (4.4)	1.72	0.690	2.498	0.078	2.133
1990–1991	244	5 918	23.1 (4.7)	1.70	0.659	2.276	0.094	2.003
1991–1992	252	5 582	23.0 (4.5)	1.71	0.653	2.399	0.094	2.020
1992–1993	266	6 760	26.1 (4.9)	1.72	0.627	2.258	0.099	1.968
1993–1994	279	7 152	26.1 (5.0)	1.73	0.667	2.357	0.093	1.984

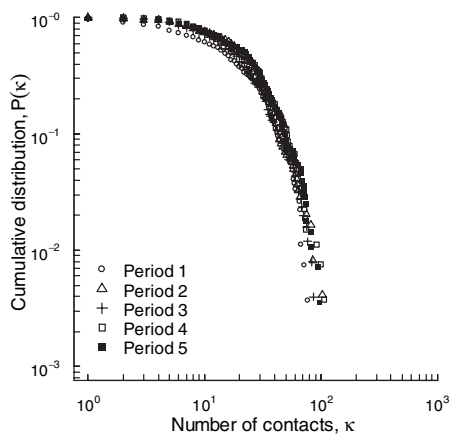


Figure 2. Log-log plot of the cumulative distributions function $P(\kappa)$ of the amount of contacts κ made by possums caught in the Castlepoint study site during the five 12-month intervals. If the distribution followed a power law, the data would tend to follow a straight line.

was compared with that expected (d_E) for a scale-free network of equivalent size, a similar difference was observed (between 31% and 45% greater; Tab. IV). These findings indicate that the observed networks show no scale-free features, instead presenting single-scale characteristics.

3.3. Effect of contact network on basic reproduction ratio

For the calculation of R_0 for TB we assumed that the duration of infectiousness D ,

followed a negative binomial distribution. The point estimate and 95% confidence interval for D was estimated by conducting 1000 bootstrap simulations where the number of trials was set to 1.25 and the daily probability of dying from TB was 7.50×10^{-3} (Fig. 1b). Based on this approach our estimate of the overall median duration of infectiousness was 4.13 months (95% CI 0.2 – 18.7 months).

Figure 3 shows the estimated value of R_0 computed from the observed network plotted as a function of a range of likely values for the transmission probability per contact, β . Superimposed on this plot are the R_0 estimates for a random network of similar size. The observed value of R_0 was significantly greater ($P < 0.05$) than the R_0 for the random network. This difference was constant over time: the observed R_0 was 1.78 (95% CI 1.77–1.79), 1.53 (95% CI 1.52–1.53), 1.52 (95% CI 1.52–1.53), 1.51 (95% CI 1.50–1.52) and 1.52 (95% CI 1.51–1.53) times greater than the equivalent random networks for each of the five 12-month periods. At the threshold value (i.e. $R_0 = 1$) the probability of infection per contact, β , between 1990 and 1994 was 0.08 (95% CI 0.06–0.11), whereas for a random network of similar size the probability of infection per contact was 0.12 (95% CI 0.09–0.16). As such, the average number of infective contacts per year per infected possum computed at the threshold value for the four 12-month networks between 1990 and 1994 were 1.95 (95% CI 1.41–2.67), 1.94 (95% CI 1.44–2.70), 1.95 (95% CI 1.44–2.61), and 1.97

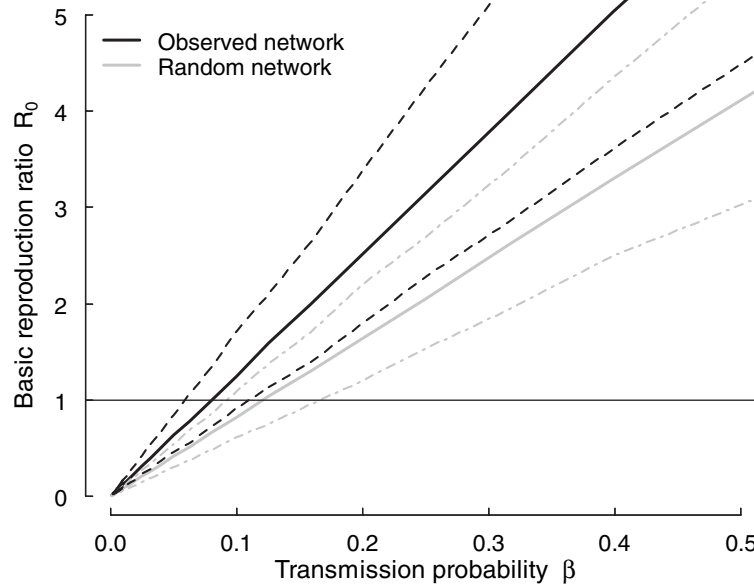


Figure 3. Estimated basic reproduction ratio R_0 for bovine tuberculosis computed for a range of plausible values of transmission probabilities (β) for the observed contact network of free-living possums for April 1990 to March 1991. For comparison, R_0 values for a network of similar size with a completely random contact pattern are shown. Solid lines represent the median values. The dashed lines represent the 95% confidence intervals around the median values. The thin horizontal line represents the threshold value $R_0 = 1$.

(95% CI 1.46–2.73), respectively, whereas the corresponding values for the random networks were 2.98 (95% CI 2.24–4.07), 2.93 (95% CI 2.17–4.01), 2.91 (95% CI 2.25–4.00), and 2.94 (95% CI 2.21–4.03). Throughout the period April 1989 to March 1990 (inclusive) a significantly larger β was observed at the threshold value ($\beta = 0.189$; 95% CI 0.143–0.245), though still remaining significantly less than that of the random network ($\beta = 0.320$; 95% CI 0.248–0.424).

4. DISCUSSION

Defining contact networks in animal populations is difficult because it requires the interactions between members of a population to be monitored and recorded for extended periods of time. While this process is easily carried out in captive animals, the logistical issues associated with data capture from large numbers of free-roaming animals in an open

population are considerable. In this study we used monthly capture data to infer direct and indirect contacts between possums in a 22-ha area that was involved in a 65-month capture-mark-recapture study. The trap-based definitions of contact assume that real contact was more likely to occur between possums in the vicinity of their trap locations. Although this may underestimate the number of contacts occurring during mating, which are associated with longer distance forays of males in particular [56], this approach is likely to provide an adequate representation of contacts associated with denning, such as den sharing and the agonistic encounters during den selection, since traps were distributed throughout the area in which possums denned¹.

¹ Pfeiffer D., The role of a wildlife reservoir in the epidemiology of bovine tuberculosis, Ph.D. thesis, IVABS, Massey University, Palmerston North, New Zealand, 1994.

Subject to the validity of our assumptions regarding what constitutes a contact, the estimated network structure in this population showed no evidence of following a random process. This is shown by the two major features of the estimated networks: the greater level of clustering and the similar geodesic distances compared with a random network of similar size. These features indicate that the observed networks are small-world, in which spread of an infection would be faster and involve a smaller proportion of individuals compared with a random network [15, 43]. Although the presence of clustering in the observed network may be (at least partly) a result of our contact definition, these features are thought to increase the likelihood of infection among members of the population. This is because: (i) it increases the likelihood of contact between possums living in the same area, and (ii) it increases the level of competition for suitable dens, which in turn increases the level of concomitant or sequential den sharing and fighting for den possession.

It has been shown using simulation methods [2, 36, 53] that constraints on individuals have a significant effect on the large-scale structure of growing networks. These constraints mean that networks grow without preferential attachment, producing either broad-scale or single-scale networks. Two particular constraints have been defined [2]: (i) the capabilities of the individuals that comprise the network (that is, the ability of individuals to form relationships with others), and/or (ii) the population size. It is worth noting that these two constraints are interrelated as both depend on the biological carrying capacity of the habitat (that is, the number of individuals an environment can support without significant negative impact on the organism and its environment [14]). As such, the single-scale features of the networks described in this study (Fig. 2) suggest that contacts between possums are dependent on the carrying capacity of the habitat, establishing an upper limit to the average number of contacts any individual possum can make [5, 61] and directly to R_0 [15, 43].

We estimated that the median percent of the population with which an individual possum made contact in each of the five 12-month intervals was between 7% and 9%. Although this constitutes a relatively small proportion of the total population, the large variability in the number of contacts, as shown in Figure 1 and Table II indicates that there are small numbers of individuals who came into contact with a relatively large proportion of the population. For example, over a one year period, one possum made contact with 130 others, representing approximately 53% of the population present in the study area during this period. Although most possums in the network are likely to have little influence on disease transmission dynamics, a small proportion in the tail of the degree distribution (Fig. 1), if infected, have the potential to have a large influence on the rate of disease spread. These individuals, termed “superspreaders” [35, 46, 70], are key determinants of disease transmission dynamics. We can only speculate on why some individual possums have a very high number of contacts. Long distance forays made by adults have the potential to be an important risk behaviour for TB transmission (see for example [21, 34]). These activities would result in an increase in the number of contacts and it would be reasonable to hypothesise that the foray distances would vary widely among individuals.

Removing highly-connected individuals from a population (i.e. those displaying superspreader characteristics) has the potential to disconnect a network and reduce the potential for infection transmission [1, 6, 15]. An important finding from our analyses is that applying such a strategy in a possum population would be inefficient since single-scale networks, unlike scale-free networks, are resilient to removal of highly connected individuals [45]. Removing highly connected possums can still be useful for controlling TB by reducing the amount of TB infection in the population and, therefore, the velocity of disease spread [46]. However, this approach implies that possums with superspreader characteristics can be reliably identified and located in a control area

which, at the time of writing, is not practical for wildlife populations.

For managing disease in wildlife populations an unbiased estimate of R_0 is of great interest since it provides an indication of the intensity of interventions (culling and/or vaccination) required to eradicate disease [4, 68]. For simplicity, estimates of R_0 for infectious diseases have mostly been based on either susceptible-infectious (SI) or susceptible-infectious-recovered (SIR) models assuming a homogeneous mixing process. Recognising that these conditions are rarely met, attempts have been made to quantify the extent and direction of the bias in estimates of R_0 in disease epidemics in animal and human populations [15, 23, 43, 50, 57]. In this study, we have extended the approach of May et al. [50] by introducing stochastic variation in the number of contacts and duration of infectiousness (Eq. (1)). This enabled the construction of a 95% confidence interval around the median estimate of R_0 , allowing comparisons to be made between the observed network and a completely random network of similar size.

Figure 3 shows R_0 as a function of transmission probability for the observed network and a completely random network of similar size for April 1990 to March 1991. Compared with the random network, the estimated values of R_0 for the observed network were between 1.51 and 1.78 times greater for given values of transmission probability. We tested the null hypothesis that the two estimates of R_0 were the same using bootstrap simulation. The null hypothesis was rejected at the alpha level of 0.05 for the entire range of transmission probabilities evaluated. These findings are consistent with those of Christley et al. [15] who compared estimates of R_0 from computer-generated small-world and random networks.

It has been shown for other diseases that infection with multi-strain microorganisms can result in a wide spectrum of responses in the host, especially with regard to the duration of infectiousness. For example, Zadocks et al. [71] showed variable clinical manifestations of naturally occurring mastitis in dairy cattle

due to *Streptococcus uberis*, when examining the association between strains and clinical characteristics of disease. If these findings are applicable to *M. bovis*, this would imply that heterogeneity in *M. bovis* sub-types recorded in this possum population² could, if unaccounted for, lead to further bias in the estimate of R_0 [67]. We believe that repetitively sampling from a distribution of survival times drawn from the naturally infected possums present in this population would account for variation in the duration of infectiousness and, therefore, the mean field value would represent the average potential for TB to spread. In this way, the wide confidence intervals shown in Figure 3 respect our hypothesis that R_0 is influenced by a heterogeneous distribution of *M. bovis* subtypes.

Since the transmission probability of TB was unknown, estimates of R_0 were calculated for a range of plausible values of β . The value of β at the threshold value (i.e., $R_0 = 1$) allowed us to evaluate the minimal amount of contact required for TB spread. Despite showing similar network features among successive years, the significantly greater estimates of R_0 for the network in the initial 12-month interval starting in April 1989 was believed to be the consequence of changes in trapping methodology that may have influenced the sampling frame¹. These changes are thought to result in a greater variability of the degree distribution compared with the successive 12-month interval networks, thereby increasing the estimates of R_0 for this period. On the basis of the networks constructed for the four 12-month periods starting in April 1990 we estimate that TB would spread if at least eight percent of contacts resulted in secondary infections, representing an average of between 1.94 and 1.97 infective contacts per year per infected possum. In contrast, the expected number of infective contacts under a random

² Jackson R., Transmission of tuberculosis caused by *Mycobacterium bovis* between possums and possums and cattle, Ph.D. thesis, IVABS, Massey University, Palmerston North, New Zealand, 1995.

process was between 2.91 and 2.98 contacts per year. In New Zealand, little information exists regarding the value of β to validate this finding. As stated by Barlow [5], “disease transmission coefficients are notoriously hard to measure in the field”, and may be subject to bias due to variable environmental conditions at the time of sampling. To overcome those problems, Barlow suggested tuning models using multiple parameter combinations to mimic the observed disease pattern. This method is somehow restricted by the limits of the actual understanding of the disease process since tuning wrong models may yield the right results for the wrong reasons [27]. We acknowledge however that our study was not designed to give an estimate of β ; it allowed only an estimation of its lower limit which, when exceeded, would enable disease to spread in the studied population.

In conclusion, we have shown that knowledge of the relationship between network topography, contact pattern, and disease transmission dynamics has the potential to enhance the understanding of disease spread in wildlife animal populations. The highly clustered heterogeneous contact pattern shown in our study indicates the need to account for heterogeneous mixing in possum TB models as advocated by Roberts [61] and Barlow [5].

Acknowledgements. We thank Simone Titus and Nigel French for the helpful discussions and useful comments. The contributions of Dirk Pfeiffer, Jenny Weston, Ian Lugton, Solis Norton, Brent Paterson and Leigh Corner who were involved in the conduct of the capture-mark-recapture study are gratefully acknowledged.

REFERENCES

- [1] Albert R., Jeong H., Barabási A.L., Error and attack tolerance of complex networks, *Nature* (2000) 406:378–382.
- [2] Amaral L.A., Scala A., Barthélemy M., Stanley H.E., Classes of small-world networks, *Proc. Natl. Acad. Sci. USA* (2000) 97:11149–11152.
- [3] Anderson R., May R., *Infectious diseases of humans: dynamics and control*, Oxford University Press, 1991.
- [4] Bacon P., MacDonald D., To control rabies: vaccinate foxes, *New Sci.* (1980) 87:640–645.
- [5] Barlow N.D., Non-linear transmission and simple models for bovine tuberculosis, *J. Anim. Ecol.* (2000) 69:703–713.
- [6] Barthélemy M., Barrat A., Pastor-Satorras R., Vespignani A., Velocity and hierarchical spread of epidemic outbreaks in scale-free networks, *Phys. Rev. Lett.* (2004) 92:178701.
- [7] Barthélemy M., Barrat A., Pastor-Satorras R., Vespignani A., Dynamical patterns of epidemic outbreaks in complex heterogeneous networks, *J. Theor. Biol.* (2005) 235:275–288.
- [8] Batagelj V., Mrvar A., Pajek-program for large network analysis, *Connections* (1998) 21:47–57.
- [9] Bivand R., Gebhardt A., Implementing functions for spatial statistical analysis using the R language, *J. Geograph. Syst.* (2000) 2:307–317.
- [10] Borgatti S., Everett M., Freeman L., UCInet version 6.137 for Windows: Software for Social Network Analysis, Analytic Technologies, Harvard, MA, USA, 2002.
- [11] Bowman A., Azzalini A., *Applied smoothing techniques for data analysis: the kernel approach with S-plus illustrations*, Oxford University Press, 1997.
- [12] Caley P., Spencer N., Cole R., Efford M., The effect of manipulating population density on the probability of den-sharing among common brushtail possums, and the implications for transmission of bovine tuberculosis, *Wildl. Res.* (1998) 25:383–392.
- [13] Caley P., Bovine tuberculosis in brushtail possums: models, dogma and data, *N. Z. J. Ecol.* (2006) 30:25–34.
- [14] Campbell N.A., Reece J.B., *Biology*, Benjamin/Cummings Publishing Company Inc., 2005.
- [15] Christley R.M., Pinchbeck G.L., Bowers R.G., Clancy D., French N.P., Bennett R., Turner J., Infection in social networks: using network analysis to identify high-risk individuals, *Am. J. Epidemiol.* (2005) 162:1024–1031.
- [16] Cohen R., Havlin S., Scale-free networks are ultrasmall, *Phys. Rev. Lett.* (2003) 90:058701.
- [17] Coleman J., Caley P., Possums as a reservoir of bovine Tb, in: Montague T. (Ed.), *The brushtail possum: biology, impact and management of an introduced marsupial*, Manaaki Whenua Press, Lincoln, New Zealand, 2000, pp. 92–104.
- [18] Corner L., Stevenson M., Collins D., Morris R., The re-emergence of *Mycobacterium bovis* infection in brushtail possums (*Trichosurus vulpecula*) after

- localised possum eradication, N. Z. Vet. J. (2003) 51:73–80.
- [19] Corner L.A., Buddle B.M., Pfeiffer D.U., Morris R.S., Vaccination of the brushtail possum (*Trichosurus vulpecula*) against *Mycobacterium bovis* infection with bacille Calmette-Guerin: the response to multiple doses, Vet. Microbiol. (2002) 84:327–336.
- [20] Corner L.A.L., Pfeiffer D.U., Morris R.S., Social-network analysis of *Mycobacterium bovis* transmission among captive brushtail possums (*Trichosurus vulpecula*), Prev. Vet. Med. (2003) 59:147–167.
- [21] Cowan P., Brockie R., Ward G., Efford M., Long-distance movements and dispersal of brushtail possums (*Trichosurus vulpecula*) on farmland, Hawke's Bay, New Zealand, Wildl. Res. (1996) 23:237–244.
- [22] Cowan P.E., Brushtail possum, in: King C.M. (Ed.), The handbook of New Zealand mammals, Oxford University Press, Auckland, New Zealand, 1990, pp. 68–98.
- [23] Crépey P., Alvarez F.P., Barthélemy M., Epidemic variability in complex networks, Phys. Rev. E (2006) 73:046131.
- [24] Croft D., Krause J., James R., Social networks in the guppy (*Poecilia reticulata*), Proc. Biol. Sci. (2004) 271:S516–S519.
- [25] Davison A., Hinkley D., Bootstrap methods and their application, Cambridge University Press, Cambridge, UK, 2003.
- [26] Day T.D., O'Connor C., Mathews L., Possum social behaviour, in: Montague T. (Ed.), The brushtail possum: the biology, impact and management of an introduced marsupial, Manaaki Whenua Press, Lincoln, New Zealand, 2000, pp. 35–46.
- [27] De Jong M.C.M., Mathematical modelling in veterinary epidemiology: why model building is important, Prev. Vet. Med. (1995) 25:183–193.
- [28] Dietz K., Transmission and control of arboviruses, in: Ludwig D., Cooke K. (Eds.), Epidemiology, SIAM, Philadelphia, PA, USA, 1975, pp. 104–121.
- [29] Dorogovtsev S., Mendes J., Evolution of networks: from biological nets to the internet and WWW, Oxford University Press, Oxford, UK, 2003.
- [30] Dublin L., Lotka A., On the true rate of natural increase, J. Am. Stat. Assoc. (1925) 150:305–339.
- [31] Efron B., Tibshirani R., An introduction to the bootstrap, Chapman & Hall, Boca Raton, Florida, USA, 1993.
- [32] Erdős P., Rényi A., The evolution of random graphs, Magyar Tud. Akad. Mat. Kutató Int. Közl. (1960) 5:17–61.
- [33] Fairweather A.A.C., Brockie R.E., Ward G.D., Possums (*Trichosurus vulpecula*) sharing dens: a potential infection route for bovine tuberculosis, N. Z. Vet. J. (1987) 35:15–16.
- [34] Fulford G.R., Roberts M.G., Heesterbeek J.A.P., The metapopulation dynamics of an infectious disease: tuberculosis in possums, Theor. Popul. Biol. (2002) 61:15–29.
- [35] Galvani A.P., May R.M., Dimensions of super-spreading, Nature (2005) 438:293–295.
- [36] Guimaraes P.J., de Aguiar M., Bascompte J., Jordano P., dos Reis S., Random initial condition in small Barabasi-Albert networks and deviations from the scale-free behavior, Phys. Rev. E (2005) 71:037101.
- [37] Heesterbeek J.A.P., A brief history of R_0 and a recipe for its calculation, Acta Biotheor. (2002) 50:189–204.
- [38] Hickling G., Clustering of tuberculosis infection in brushtail possum populations: implications for epidemiological simulation models, in: Griffen F., de Lisle G. (Eds.), Tuberculosis in wildlife and domestic animals, University of Otago, Dunedin, New Zealand, 1995, pp. 276–279.
- [39] Jackson R., de Lisle G., Morris R., A study of the environmental survival of *Mycobacterium bovis* on a farm in New Zealand, N. Z. Vet. J. (1995) 43:346–352.
- [40] Jackson R., The role of wildlife in *Mycobacterium bovis* infection of livestock in New Zealand, N. Z. Vet. J. (2002) 50:49–52.
- [41] Ji W., Sarre S., Craig J., Clout M., Denning behavior of common brushtail possums in populations recovering from density reduction, J. Mammal. (2003) 84:1059–1067.
- [42] Ji W., White P.C.L., Clout M., Contact rates between possums revealed by proximity data loggers, J. Appl. Ecol. (2005) 42:595–604.
- [43] Keeling M., The implications of network structure for epidemic dynamics, Theor. Popul. Biol. (2005) 67:1–8.
- [44] Li L., Alderson D., Tanaka R., Doyle J., Willinger W., Towards a theory of scale-free graphs: definition, properties, and implications, Internet Math. (2005) 2:431–523.
- [45] Liljeros F., Edling C.R., Amaral L.A.N., Stanley H.E., Åberg Y., The web of human sexual contacts, Nature (2001) 411:907–908.
- [46] Lloyd-Smith J., Schreiber S., Kopp P., Getz W., Superspreading and the effect of individual variation on disease emergence, Nature (2005) 438:355–359.

- [47] Lusseau D., The emergent properties of a dolphin social network, *Proc. Biol. Sci.* (2003) 270:S186-S188.
- [48] Lusseau D., Newman E., Identifying the role that animals play in their social networks, *Proc. Biol. Sci.* (2004) 271:S477-S281.
- [49] Lusseau D., Wilson B., Hammond P., Grellier K., Durban J., Parsons K., et al., Quantifying the influence of sociality on population structure in bottlenose dolphins, *J. Anim. Ecol.* (2006) 75:14–24.
- [50] May R.M., Gupta S., McLean A.R., Infectious disease dynamics: what characterizes a successful invader?, *Philos. Trans. R. Soc. Lond. B Biol. Sci.* (2001) 356:901–910.
- [51] May R.M., Lloyd A.L., Infection dynamics on scale-free networks, *Phys. Rev. E* (2001) 64:066112.
- [52] Mollema L., Koene P., de Jong M., Quantification of the contact structure in a feral cattle population and its hypothetical effect on the transmission of bovine herpesvirus, *Prev. Vet. Med.* (2006) 77:161–179.
- [53] Mossa S., Barthelemy M., Eugene Stanley H., Nunes Amaral L., Truncation of power law behavior in “scale-free” network models due to information filtering, *Phys. Rev. Lett.* (2002) 88:138701.
- [54] Neal P., SIR epidemics on a Bernoulli random graph, *J. Appl. Probab.* (2003) 40:779–782.
- [55] Norton S., Corner L., Morris R., Ranging behaviour and survival duration of wild brushtail possums (*Trichosurus vulpecula*) infected with *Mycobacterium bovis*, *N. Z. Vet. J.* (2005) 53:293–300.
- [56] Paterson B., Morris R., Weston J., Cowan P., Foraging and denning patterns of brushtail possums, and their possible relationship to contact with cattle and the transmission of bovine tuberculosis, *N. Z. Vet. J.* (1995) 43:281–288.
- [57] Potterat J.J., Zimmerman-Rogers H., Muth S.Q., Rothenberg R.B., Green D.L., Taylor J.E. et al., *Chlamydia* transmission: concurrency, reproduction number, and the epidemic trajectory, *Am. J. Epidemiol.* (1999) 150:1331–1339.
- [58] Ramsey D., Spencer N., Caley P., Efford M., Hansen K., Lam M., Cooper D., The effects of reducing population density on contact rates between brushtail possums: implications for transmission of bovine tuberculosis, *J. Appl. Ecol.* (2002) 39:806–818.
- [59] Ramsey D., Cowan P., Mortality rate and movements of brushtail possums with clinical tuberculosis (*Mycobacterium bovis* infection), *N. Z. Vet. J.* (2003) 51:179–185.
- [60] Ramsey D., Coleman J., Coleman M., Horton P., The effect of fertility control on the transmission of bovine tuberculosis in wild brushtail possums, *N. Z. Vet. J.* (2006) 54:218–223.
- [61] Roberts M., The dynamics of bovine tuberculosis in possum populations, and its eradication or control by culling or vaccination, *J. Anim. Ecol.* (1996) 65:451–464.
- [62] Rowlingson B., Diggle P., Splan: spatial point pattern analysis code in S-PLUS, *Comput. Geosci. UK* (1993) 19:627–655.
- [63] Smith G., Models of *Mycobacterium bovis* in wildlife and cattle, *Tuberculosis* (2001) 81:51–64.
- [64] Snijders T., Borgatti S., Non-parametric standard errors and tests for network statistics, *Connections* (1999) 22:161–170.
- [65] Wasserman S., Faust K., Social network analysis: methods and applications, Cambridge University Press, Cambridge, UK, 1994.
- [66] Watts D.J., Strogatz S.H., Collective dynamics of “small-world” networks, *Nature* (1998) 393:440–442.
- [67] Wearing H.J., Rohani P., Keeling M.J., Appropriate models for the management of infectious diseases, *PLoS Med.* (2005) 2:e174.
- [68] Wierup M., The Swedish canine parvovirus epidemic – an epidemiological study in a dog population of defined size, *Prev. Vet. Med.* (1983) 1:273–288.
- [69] Wittemyer G., Douglas-Hamilton I., Getz W., The socioecology of elephants: analysis of the processes creating multitiered social structures, *Anim. Behav.* (2005) 69:1357–1371.
- [70] Yorke J., Hethcote H., Nold A., Dynamics and control of the transmission of gonorrhoea, *Sex. Transm. Dis.* (1978) 5:51–56.
- [71] Zadocks R.N., Gillespie B.E., Barkema H.W., Sampimon O.C., Oliver S.P., Schukken Y.H., Clinical, epidemiological and molecular characteristics of *Streptococcus uberis* infections in dairy herds, *Epidemiol. Infect.* (2003) 130:335–349.