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Network structure and prevalence of *Cryptosporidium* in Belding's ground squirrels

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Abstract Although pathogen transmission dynamics are profoundly affected by population social and spatial structure, few studies have empirically demonstrated the population-level implications of such structure in wildlife. In particular, epidemiological models predict that the extent to which contact patterns are clustered decreases a pathogen's ability to spread throughout an entire population, but this effect has yet to be demonstrated in a natural population. Here, we use network analysis to examine patterns of transmission of an environmentally transmitted parasite, *Cryptosporidium* spp., in Belding's ground squirrels (*Spermophilus beldingi*). We found that the prevalence of *Cryptosporidium* was negatively correlated with transitivity, a measure of network clustering, and positively correlated with the percentage of juvenile males. Additionally, network transitivity decreased when there were higher percentages of juvenile males; the exploratory behavior demonstrated by juvenile males may have altered the structure of the network by reducing clustering, and low clustering was associated with high prevalence. We suggest that juvenile males are critical in mediating the ability of *Cryptosporidium* to spread through colonies, and thus may function as "super-spreaders." Our results demonstrate the utility of a network approach in quantifying mechanistically how differences in contact patterns may lead to system-level differences in infection patterns.

Keywords Social networks · *Cryptosporidium* · Ground squirrels · Pathogen transmission · Infection patterns · Clustering · Wildlife disease

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Introduction

Processes underlying the transmission of pathogens through populations are complex and highly dependent on host behavior, yet traditional approaches have ignored behavior partly due to the computational sophistication required to incorporate behavioral complexity. Classical compartmental models of the spread of infectious disease assume that individuals within populations mix homogenously, and that the probability of contact is equal for every pair of individuals in the population (Anderson and May 1992; McCallum et al. 2001). In reality, however, contact patterns are highly heterogeneous and an animal's contact rate with infected individuals is dependent on local patterns of interaction. Local spatial and social structures in populations create heterogeneity in transmission patterns (Keeling and Eames 2005; Bansal et al. 2007; Otterstatter and Thomson 2007; Perkins et al. 2008).

Network theory provides a set of tools for analyzing heterogeneity in contact patterns. A network consists of a set of

nodes (individuals) that are interlinked based on some criterion (Wasserman and Faust 1994; Croft et al. 2008). In practice, criteria for linking edges may include measures of proximity, social interactions, or shared space use. Models that account for heterogeneity in connectivity tend to result in epidemics with slower growth rates, fewer numbers of secondary infections for each infected individual (R_0), and lower overall prevalence as compared to traditional mass-action models (Keeling 2005; Keeling and Eames 2005; Turner et al. 2008). This occurs primarily because the network becomes locally depleted of susceptible individuals, especially in highly clustered networks (Keeling 1999; Keeling and Eames 2005). In addition, heterogeneity in the number of contacts each individual has may alter transmission dynamics if few key individuals are disproportionately involved in transmission (Lloyd-Smith et al. 2005).

Transmission is more rapid through highly dense networks in which each node has a high mean number of contacts. However, epidemics in networks of equal density can exhibit profoundly different behavior depending on the degree of clustering (Keeling 2005; Ames et al. 2011). Generally, as clustering increases, both R_0 and the final size of the epidemic decrease (Keeling 1999; Newman 2003; Turner et al. 2008; Wu and Liu 2008; Badham and Stocker 2010; Ames et al. 2011). For example, animals A, B, and C are all in contact with one another in a clustered network. If animal A infects B and B infects C, the number of susceptible individuals that C is able to infect is reduced because two of its contacts are already infected. Thus, the network becomes locally depleted of susceptible individuals. By incorporating aspects of social structure, such as clustering, models demonstrate how knowledge of network structure can help us to predict population-level epidemic dynamics from individual-level behavior.

Few field studies have incorporated network data with empirical data on pathogen presence. Thus, far studies have found that individuals generally have a higher risk of infection if they are better connected in the network (Corner et al. 2003; Otterstatter and Thomson 2007; Godfrey et al. 2009; Fenner et al. 2011). Individuals that are directly linked to a greater number of animals tend to be more at risk (Corner et al. 2003; Godfrey et al. 2010), although some authors show that only certain types of interactions or only contact with infected animals impact risk (Otterstatter and Thomson 2007; Drewe 2009; Porphyre et al. 2011). In general, these studies have looked at the effect of network measures on an individual's risk of disease, but few of them have looked at the implications of network structure at a population level. One exception is found in Otterstatter and Thomson (2007), who demonstrated that infection spreads more rapidly though denser networks. Although mathematical models predict this relationship, density does not account for other aspects of structure frequently shown to be critical for predicting pathogen dynamics, such as clustering.

Here, we use a network approach to investigate infection patterns of an enteric protozoal parasite, *Cryptosporidium* spp. in two colonies of Belding's ground squirrels (*Spermophilus beldingi*). Cryptosporidiosis is endemic in populations of Belding's ground squirrels in the Sierra Nevada Mountains, and parasites within this genus are environmentally persistent and have zoonotic potential (Zu et al. 1992). In California ground squirrels (*Spermophilus beecheyi*), *Cryptosporidium* prevalence peaked seasonally as a consequence of increased population density and an influx of immunologically naïve juveniles (Atwill et al. 2004). In this study, we first examine the association between network structure and prevalence of *Cryptosporidium* spp. and then investigate the impact of juvenile emergence on social network structure and the spread of infection. We also analyze risk factors influencing the probability that an individual acquires the infection.

Methods

Study site and population

The Belding's ground squirrel is a colony-dwelling social rodent primarily found in alpine regions of California, which includes the Sierra Nevada and extends north to the Oregon border. Squirrels are active from May through September, though adults emerge from hibernation weeks to months before juveniles emerge from their natal burrows (Michener 1984). When juveniles do emerge at about 3 to 4 weeks old, their numbers cause a rapid increase in population density. Adult females maintain exclusive use of a home burrow system, though they occupy overlapping home ranges (McLean 1984).

Two Belding's ground squirrel colonies were included in this study. Both colonies (A and B) were located within Tuolumne Meadows, Yosemite National Park, California, at an elevation of approximately 8,600 ft. Because the two colonies were separated by distance (~5 mi) and geographic barriers (Tuolumne River), there was no movement of individuals between colonies. Sampling began in late June 2004 and continued through August 2004. One hundred fifty-eight Belding's ground squirrels were trapped and identified in this study (27 adult females, 63 juvenile females, 17 adult males, and 51 juvenile males). All squirrels trapped throughout the season were included in the study.

Live-trapping was conducted at each colony approximately twice per week. Traps were placed at all active burrow entrances at each site. This produced a grid of 40 traps spaced at approximately 50 m intervals in colony A, and 32 traps spaced at 55 m intervals in colony B. Traps were cleaned between captures and disinfected nightly to avoid cross-contamination of samples. When trapped, squirrels were temporarily restrained, weighed, and ear-tagged. The age of all subjects was determined by weight. Fecal samples were collected from each new and re-

trapped individual as often as every 3 days and analyzed for the presence and abundance of *Cryptosporidium* spp. (Atwill et al. 2001). Traps captured only one squirrel at a time. Individual squirrels were trapped on average 11.1±0.71 SE times during the 8-week study period.

Enumeration of *Cryptosporidium* spp.

Oocysts were concentrated using sieved fecal suspension, and direct immunofluorescent microscopy was used to detect and enumerate *Cryptosporidium* spp. oocysts, as previously described (Atwill et al. 2001). Final oocyst counts were adjusted for percent recovery, determined previously to be ~10 % for oocyst concentrations <1,000 per gram feces and ~16.5 % for oocyst concentrations >1,000 per gram feces (Atwill et al. 2001). Enumeration procedures were performed at the Veterinary Medical Teaching and Research Center of the University of California, Davis School of Veterinary Medicine.

Contact patterns

Two individuals were considered in "contact" with one another if they were trapped in the same trap on the same day (only one squirrel was trapped at a time, but traps were reset throughout the day after each capture). This measure of association has been used in other studies of disease transmission in rodents and demonstrates shared space use (Perkins et al. 2009). Shared space use increases both the probability of environmental transmission and also the likelihood of social interaction. Prevalence was defined as the proportion of individuals with positive oocyst counts. Prevalence of *Cryptosporidium* was calculated for the entire population and for exposed versus non-exposed squirrels. For this comparison, an exposed individual was defined as any squirrel that was previously in contact with an infected squirrel at a previous time point. Non-exposed squirrels were either those that had not yet been or were never documented as in contact with an infected squirrel. Individual squirrels may appear in both the non-exposed and exposed groups if data on their infection status exist for both before and after their first recorded contact with an infected individual. An exposed animal may never reenter the non-exposed group even if many weeks have elapsed with no subsequent exposures.

Longitudinal trends

To investigate how past contact patterns affected current infection status, we subdivided the 10 weeks of data into 2-week periods. Two weeks was sufficient time to obtain a reasonable sample size for network analysis, yet short enough to capture the dynamic nature of contact patterns. Although not known for ground squirrels, peak fecal oocyst shedding occurs about

2–4 weeks after exposure for other rodent species (Benarska et al. 2003), though infection is detectable in the intestines of euthanized mice 7 days after experimental inoculation (Hou et al. 2004). Thus, we decided to investigate the effect of contact rates during a 2-week period, termed the "contact period," on the infection patterns observed in the following 2 weeks, the "response period."

Non-overlapping contact periods were used in network construction. The first week was excluded because of low trapping effort (week of arrival to study site). Trapping effort was otherwise similar across trapping weeks. Because 4 weeks of data collection constituted a complete time series, we were only able to define three sets of complete time series. An additional period was constructed using trapping data from August 8, 2004 to August 21, 2004 to examine trends in network structure, but there was no corresponding data available on *Cryptosporidium* infection due to the ending of the field season.

We investigated how overall network structure during the contact period affected prevalence in the response period. Individual squirrels were linked in the network if they were in contact at least once during the contact period, creating an unweighted contact network. Separate networks were constructed using NetMiner (NetMiner 2.6, Cyram Corporation, Seoul, Korea) for each colony and contact period for a total of eight networks. We analyzed the effect of colony-level attributes on prevalence using logistic regression models for grouped data in Stata (Stata 9, Stata Corporation, College Station, TX). Here, prevalence was defined as the percentage of individuals with positive oocyst counts in the response period. Each model included period to account for temporal shifts in prevalence. Colony was included as a random effect in order to control for potentially correlated data due to repeated sampling. Other covariates were included singly because the small number of networks prevented the construction of more complex models; AIC was used to compare models. Covariates included were group size (number of individuals trapped for that site and contact period), trapping days (number of days spent trapping during the 2-week contact period), percent juveniles (percentage of group members that were juveniles), percent juvenile males, percent emerging juveniles (newly emerged juveniles that were trapped for the first time during the contact period), and prevalence during the contact period. Five measures of network structure were also examined: transitivity, density, betweenness centralization, closeness centralization, and degree centralization.

Transitivity, also known as the global clustering coefficient, was defined as the number of triangles in the network (A is linked to B, B is linked to C, and C is also linked to A) relative to the number of triplets (e.g., A is linked to B, B is linked to C, but C is not linked to A). Density is the number of links that exist in the network divided by the total number

possible. Transitivity and density are reported as percentages. From an epidemiological perspective, high density networks theoretically result in more infected individuals, while highly transitive networks result in fewer infected individuals (Ames et al. 2011). Degree is the number of individuals that the focal node is directly connected to. This is the only network measure we used that incorporated connection strength between individuals; individuals received higher scores if they have been trapped multiple times with the same individual. Betweenness refers to the number of paths that pass through the focal individual if the shortest path is traced between all other pairs of individuals. Closeness quantifies the distance between the focal node and all other nodes in the network (Wasserman and Faust 1994). Centralization for any network measure (degree, betweenness, and closeness) essentially measures the extent to which the network is structured around a few core individuals (Wasserman and Faust 1994). High centralization can facilitate pathogen spread because core individuals can rapidly transmit pathogens to many other individuals. However, pathogen spread can be slowed if these core individuals remain uninfected (Valente 2010).

Individual risk factors

In addition to examining how network structure impacts overall levels of infection in colonies, we were also interested in how an individual's location in the network affected its likelihood of acquiring *Cryptosporidium*. To determine which factors increase an individual's risk of becoming infected, we performed mixed effects logistical regression on the probability that an individual would be infected in the response period. An individual was considered to be infected if it had a positive oocyst count in any fecal samples collected from it during that period. Permutation-based methods, which are often used in the statistical analysis of network data (Croft et al. 2011), were not utilized here because the response variable was not based on relational data. Covariates included infection state during the contact period, sex, age (juvenile or adult), degree, infected degree, closeness, and betweenness. Degree and infected degree differ in that degree includes all an individual's contacts during the contact period while infected degree only includes those contacts that were infected. To account for the possibility that exposure prior to the contact period may still influence infection risk, we also included a covariate termed "precontact infected degree," which refers to the individual's infected degree in the third and fourth week prior to the response period. Colony, individual ID, and period were included as random effects. Candidate models were assessed using AIC, and the simplest model that was <2 ΔAIC from the model with the lowest AIC was selected. Models <2 ΔAIC from each other can be considered to explain

equivalent amounts of variation in the data (Burnham and Anderson 2002).

We also developed a measure to quantify an individual's tendency to explore. Exploratoriness was defined as the diversity of traps a squirrel was caught in relative to the expected diversity for a squirrel being caught a given number of times. A power regression line ($y = 1.02 * x^{0.74}$) was fitted that related the number of different traps squirrels were captured in to the total number of times trapped. From this regression equation, we calculated the expected diversity of traps given total number of times trapped for each squirrel. A residual was then calculated from the observed diversity of traps minus the expected diversity generated from the regression line. Squirrels that had positive residuals relative to the regression line were considered to be more exploratory (weighted by the magnitude of residual) and squirrels with negative residuals were considered less exploratory. These exploratory measures were calculated for each squirrel in each time period.

Results

The overall prevalence (percentage of individuals that had positive oocyst counts at least once during the summer) of *Cryptosporidium* spp. in the two colonies of Belding's ground squirrels was 22.9±1.5 %. The prevalence in squirrels that had been exposed at any point in the study period to infected individuals was 27.7±2.2 %, while the prevalence in squirrels that experienced no documented exposure was 9.7±2.2 % ($z = 4.85$, $p < 0.001$). Across the field season, prevalence in both colonies A and B increased with each 2-week period (ß=0.63, $p < 0.01$) and was higher at colony A than B (ß=−1.15, $p < 0.01$, Fig. 1). The percentage of juveniles increased moderately over time as they emerged from their natal burrows (Fig. 2).

From an individual perspective, risk of infection during the response period was positively correlated with infected degree during the contact period (ß=0.21, $p = 0.014$). Juveniles were significantly more likely to become infected than adults (ß=2.96, $p < 0.001$). Models that contained additional individual-level covariates explained little additional variation in the data. Indeed, an individual's overall degree, precontact infected degree (in the third and fourth week prior to the response period), closeness, betweenness, sex, and infection state during the contact period were not significantly associated with infection status.

Controlling for colony and period, the factor that explained the most variation in the prevalence of *Cryptosporidium* spp. at the colony-level was percent juvenile males, followed by transitivity in the contact period (Table 1). Groups with greater numbers of juvenile males in the contact period exhibited higher prevalence in the response period (Fig. 3a). Although
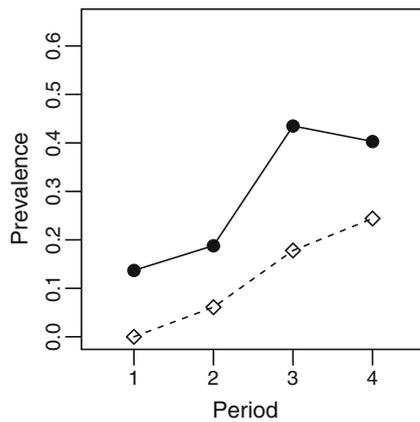
Fig. 1 Changes in the prevalence (±SE) of *Cryptosporidium* spp. in Belding's ground squirrels over time in colony A (*solid line*) and B (*hashed line*)

the percentage of adult males and juvenile females were significant in univariate analyses, these factors became non-significant once the percentage of juvenile males was included in multivariate models. Prevalence in the response period was also negatively correlated with transitivity in the contact period (Fig. 3b). Group size was also positively correlated with prevalence (Table 1) and was not correlated with percent juveniles ($\beta = 0.01$, $p = 0.28$). Group size peaked in period 2, while prevalence peaked later for both colonies. All other models explained much less variance than these three factors (ΔAIC >4.0 relative to best model).

Because juvenile males emerged as the only age class whose numbers correlated with prevalence, we examined the effect of
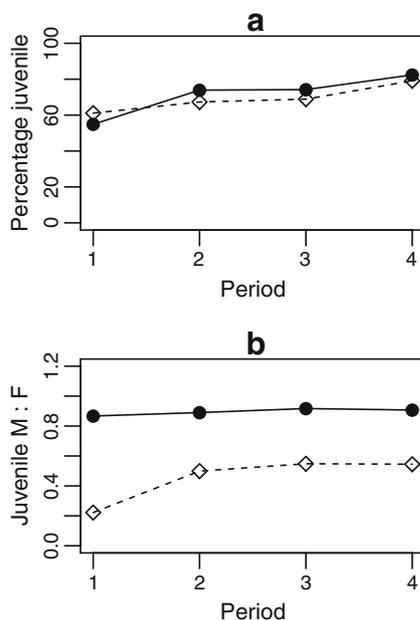

Fig. 2 **a** Percentage of population that were juveniles in each colony across 2-week time periods. **b** Juvenile sex ratio across time periods. Colonies A and B are indicated by *solid* and *hashed lines*, respectively

juvenile males on network structure. Although the overall proportion of juveniles was approximately equal across colonies (Fig. 2a), the juvenile sex ratio was considerably different in the two colonies ($\beta = -0.64$, $p < 0.01$). Colony A had an approximately equal number of juvenile males and females (1:1 or 1.0), while colony B had only half as many juvenile males as females (Fig. 2b). As the percentage of juvenile males in a group increased, the network became more dense ($\beta = 0.013$, $p < 0.01$) and less clustered ($\beta = -0.007$, $p < 0.01$, Fig. 3c). This correlation with network structure was only significant for juvenile males (effect of percent juveniles on density and transitivity, $p > 0.4$) (Table 2). In addition, males were more exploratory than females ($\beta = 0.21$, $p < 0.03$) and juveniles were more exploratory than adults ($\beta = 0.19$, $p < 0.05$). Juvenile males showed an increase in exploratory behavior over the summer ($\beta = 0.33$, $p < 0.02$) whereas all other age/sex classes did not.

Discussion

This study demonstrates that trapping networks can explain transmission patterns in pathogens with indirect transmission. Individuals that were in contact with infected squirrels were 2.84 times (cumulative risk ratio) more likely to become infected compared to squirrels that did not come in contact with infected individuals. Even when we restricted the analysis by assuming a 2–4 week response period in the longitudinal data, exposed individuals were 1.5 times more likely to become infected in the subsequent time period as compared to individuals that experienced no documented exposure. The difference between the two cumulative risk ratios likely results from our assumption about the response period. First, in the longitudinal analysis, individuals that were exposed during the contact period may not have begun shedding oocysts until after the response period, causing them to be erroneously classified as uninfected. Second, individuals that had no contact with infected squirrels during the contact period may have been exposed before the contact period, causing them to be erroneously classified as unexposed. Since *Cryptosporidium* is primarily environmentally transmitted, the networks constructed in this study likely quantify the significance of shared space use. However, we cannot exclude social interaction (i.e., direct contact transmission) as a mode of transmission, especially considering that traps were located at burrow entrances.

Individual infection risk was best predicted by the number of infected contacts and the age of the animal. Juveniles were more likely to be infected, a pattern commonly observed across many *Cryptosporidium* host species (Zu et al. 1992; Atwill et al. 2001). While other studies have demonstrated that degree correlates with infection risk (Corner et al. 2003; Godfrey et al. 2009), our results concur with the results of Otterstatter and Thomson (2007) and Porphyre et al. (2011)

Table 1 Correlations between colony-level attributes and prevalence (*p value <0.05). Models were fitted using logistic regressions for grouped data. Each factor represents a different model that included colony as a random effect and sampling period. Sampling period, which referred to the 2-week period in which fecal samples were collected, was significant in all models

Model	ß	SE	ΔAIC
% Juvenile males	0.07	0.003	0.0*
Transitivity	−0.08	0.000	0.4*
Group size	0.04	0.01	1.0*
Current prevalence	3.30	1.15	4.5*
% Juveniles	0.07	0.03	6.4
Density	−0.003	0.001	9.0
Mean degree	−1.82	0.84	9.2
Mean betweenness	−20.43	0.78	10.0*
% Emerging juveniles	−0.01	0.02	10.3
Betweenness centralization	−0.00	0.00	10.4
Degree centralization	−0.004	0.003	10.1
Trapping days	0.343	0.443	21.5
Null	n/a	n/a	25.6

that the number of infected contacts is much more important than the overall number of contacts. Degree may not serve as a useful proxy for infected degree because it does not necessarily correlate with infected degree. Indeed, individuals with higher degree sometimes show a reduced likelihood of becoming infected (Cohen et al. 1997; Porphyre et al. 2011). Similarly, the types of interactions that an animal engages in may be much more important than the overall amount of interaction (Drewe 2009). Infected individuals may also exhibit altered interaction patterns due to changes in their own behavior or the behavior of conspecifics towards them (Hawley et al. 2011). These studies taken together suggest that caution should be exercised when using overall degree as a proxy for contact with infected individuals.

While our analysis showed that network measures beyond infected degree provide little explanatory value in predicting

individual risk, complex network measures were useful in predicting population-level patterns on infection. One of the most important factors correlated with colony-level spread of *Cryptosporidium* was transitivity, a measure of local clustering. Theoretical models demonstrate that high clustering generally reduces the ability of a disease to spread through populations (Keeling 1999; Newman 2003; Turner et al. 2008; Wu and Liu 2008; Badham and Stocker 2010; Ames et al. 2011), particularly in networks with intermediate densities (Turner et al. 2008; Ames et al. 2011). Yet, this effect has not been demonstrated in an empirical study of wildlife. Clustering refers to the degree to which nodes with a neighbor in common are likely to be connected themselves. Transmission is inhibited in a more transitive network because the network becomes locally saturated with infected individuals, even if susceptible individuals exist elsewhere. This sort of clustering can prevent wider dissemination of infection.

On a colony level, the other factor that was strongly correlated with prevalence was percent juvenile males. Populations with a high percentage of juveniles can be expected to have a higher prevalence of infection simply due to the presence of numerous immunologically naïve animals (Zu et al. 1992). Infected juveniles also shed on average 36 times higher concentration of oocysts than infected adults (unpublished data). However, if the presence of naïve individuals was a main driver influencing prevalence, then we would expect prevalence to be correlated with either the number of newly emerged juveniles in the population (percent new juveniles) or the total number of juveniles (percent juveniles). Neither of these measures was significant (Table 1). In addition, we would expect both colonies to have similar prevalence given that they had similar proportions of juveniles (Fig. 2a), yet starkly higher prevalence was observed in colony A. One key difference between the colonies was in their juvenile sex ratios. Colony A had a nearly even juvenile sex ratio, while colony B had nearly twice as many juvenile females as males (Fig. 2b).
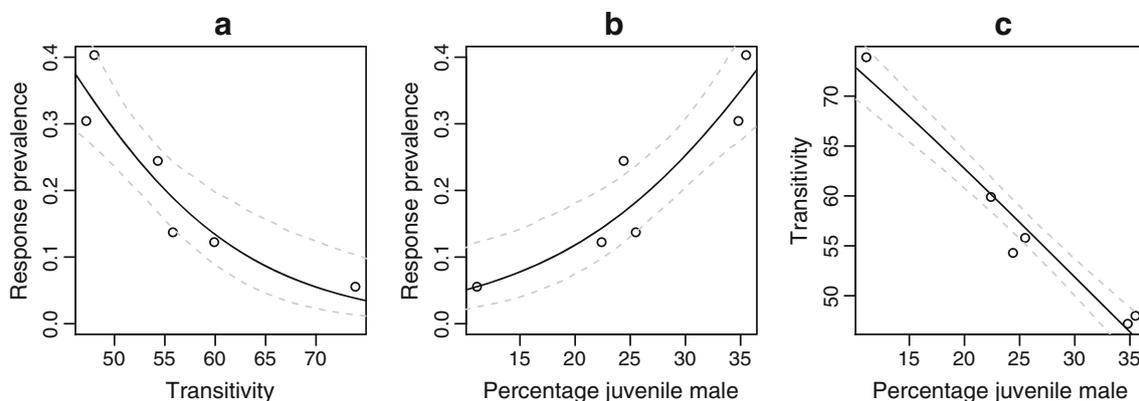

Fig. 3 Correlations between response prevalence and two colony-level attributes, **a** transitivity and **b** percent juvenile males, and **c** the relationship between juvenile males and transitivity. Model predictions with 95 % confidence intervals are plotted

Table 2 Correlations between percent juveniles in a colony and measures of network structure (transitivity and density)

	ß	SE	p value
TRANSIVITY			
% Juveniles	−0.003	0.003	0.45
% Juvenile males	−0.007	0.003	0.007
DENSITY			
% Juveniles	−0.006	0.003	0.66
% Juvenile males	0.013	0.003	<0.001

Controlling for colony and period, the percentage of juvenile males explained more variance in prevalence than percent juveniles or the percentage of any other age class.

We suggest that the importance of juvenile males arises from their behavior and their role as dispersers. Juvenile males are more socially active than females (Murie and Michener 1984). Moreover, juvenile males exhibit more sociosexual play, involving higher rates of physical contact and specifically fecal–oral contact than juvenile females (Nunes et al. 1999), creating greater opportunities for direct transmission. Perhaps more importantly, juvenile males were the most exploratory age class and their exploration increased over the summer. This is likely because they are the dispersing sex, and dispersal usually occurs within 3 to 12 weeks postemergence (Holekamp 1984). Dispersal is usually preceded by an exploratory period where the juvenile may roam long distances from the natal burrow (Holekamp 1984). The consequence of this exploration for network structure may be to link clusters of individuals that may otherwise have been poorly connected, creating more pathways for transmission to occur. Indeed, networks with a high percentage of juvenile males had lower clustering (Fig. 3). While percent juvenile males were also positively correlated with network density, density was not as strongly correlated with prevalence as transitivity (Table 1). Thus, from an epidemiological perspective, the exploratory behavior typical of juvenile males is significant to transmission dynamics in that it creates networks with reduced levels of clustering, which is in turn correlated with high prevalence. The effect of juvenile males on network connectivity is readily apparent even to visual inspection (Fig. 4). Therefore, we argue that juvenile males may be critical in mediating the ability of *Cryptosporidium* to spread because their behavior as the dispersing sex alters the structure of the contact network.

This study demonstrates how network theory can be utilized to identify classes of individuals that may be critical for pathogen spread. Other studies suggest that certain types of individuals are especially important in the maintenance and spread of pathogens. Adult males have been recognized in both empirical and network modeling studies as playing a critical role in disease spread (Ferrari et al. 2003; Perkins

et al. 2008; Grear et al. 2009). It has been experimentally demonstrated that males with higher testosterone maintain more connections in rodent populations (Grear et al. 2009), and disassortative mixing of sexes and extreme sex biases in transmission are necessary for network models to accurately match observed prevalence (Perkins et al. 2008). Furthermore, when the infectiousness of adult male or female mice was experimentally reduced in replicated study populations, reducing the parasitic load of males resulted in population-wide declines in prevalence. A similar effect was not observed when females were targeted. While the authors did discuss possible
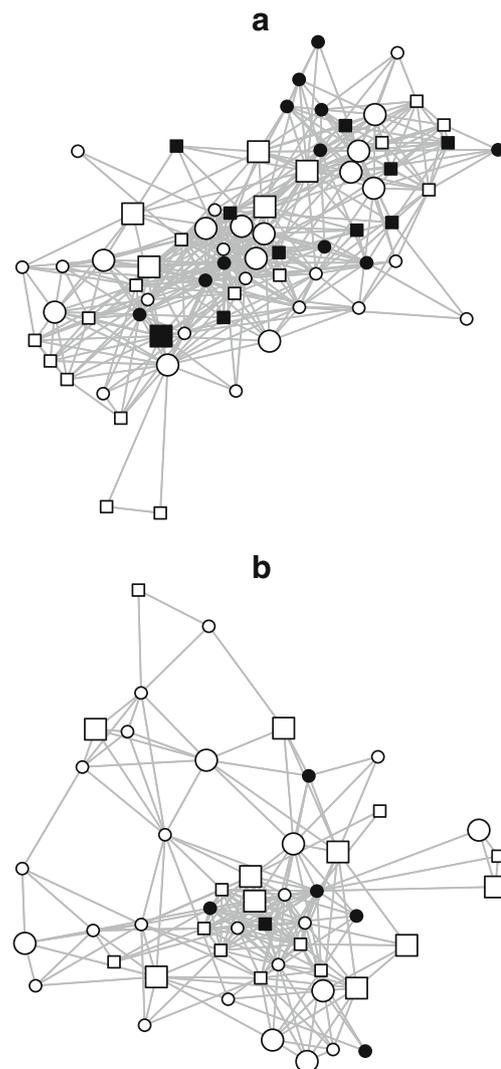

Fig. 4 Contact networks for colony A (*N*=69) and B (*N*=49) during the second 2-week time period. *Squares* and *circles* represent males and females, respectively. *Large and small nodes* denote adults and juveniles, respectively. *Black nodes* are those that tested positive for *Cryptosporidium* during the contact period. Both networks had similar densities (18.5 and 18.7 % for A and B, respectively) and similar percentages of juveniles (73.9 and 67.3 %). Colony A had a greater percentage of juvenile males (34.8 versus 22.4 % for A and B, respectively) and also lower transitivity (47.2 versus 59.9 %)

immunological sex differences, they emphasized that sex differences in behavior may have played a large role in explaining this pattern (Ferrari et al. 2003). It seems likely that the application of network theory would cast some light on the mechanism explaining their results. However, this study did not concurrently measure pathogen presence and social patterns in their populations. Our results combine data on spatial/social patterns with concurrent data on pathogen presence to address this issue.

Finally, this study demonstrates the importance of network structure in predicting the impact of pathogens at the group or population level in wildlife. It would have been difficult to explain colony-level differences in the ability of the infection to spread without characterizing the network. Even with our small sample size, network analysis was able to capture differences in social structure that were correlated with variation in prevalence. Indeed, it was possible to identify types of networks that correlated with higher prevalence, quantify how these measures vary between colonies and across time, and identify classes of individuals that may contribute disproportionately to transmission due to their possible influence on network structure. While social networks have proven to be a useful tool in predicting individual risk, we believe that their true value lies in their ability to quantify fine-scale differences in contact patterns that lead to emergent system-level differences in infection patterns.

Ethical standards The experiments described herein comply with the current laws of the USA.

References

Ames GM, George DB, Hampson CP, Kanerek AR, McBee CD, Lockwood DR, Achter JD, Webb CT (2011) Using network properties to predict disease dynamics on human contact networks. Proc R Soc Lond B 278:3544–2550

Anderson RM, May RM (1992) Infectious diseases of humans. Oxford University, Oxford

Atwill ER, Camargo SM, Phillips R, Alonso LH, Tate KW, Jensen WA, Bennet J, Little S, Salmon TP (2001) Quantitative shedding of two genotypes of *Cryptosporidium parvum* in California ground squirrels (*Spermophilus beecheyi*). Appl Environ Microbiol 67:2840–2843

Atwill ER, Phillips R, Pereira MGC, Li X, McCowan B (2004) Seasonal shedding of multiple *Cryptosporidium* genotypes in California ground squirrels (*Spermophilus beecheyi*). Appl Environ Microbiol 70:6748–6752

Badham J, Stocker R (2010) The impact of network clustering and assortivity on epidemic behaviour. Theor Popul Biol 77:71–75

Bansal S, Grenfell BT, Meyers LA (2007) When individual behaviour matters: homogeneous and network models in epidemiology. J R Soc Interface 4:879–891

Benarska M, Bajer A, Kulis K, Sinski E (2003) Biological characterisation of *Cryptosporidium parvum* isolates of wildlife rodents in Poland. Ann Agric Environ Med 10:163–169

Burnham KP, Anderson DR (2002) Model selection and multimodal inference: a practical information-theoretic approach. Springer, New York

Cohen S, Doyle WJ, Skoner DP, Rabin BS, Gwaltney JMJ (1997) Social ties and susceptibility to the common cold. JAMA-J Am Med Assoc 277:1940–1944

Corner LAL, Pfeiffer DU, Morris RS (2003) Social-network analysis of *Mycobacterium bovis* transmission among captive brushtail possums (*Trichosurus vulpecula*). Prev Vet Med 59:147–167

Croft DP, James R, Krause J (2008) Exploring animal social networks. Princeton University, Princeton

Croft DP, Madden JR, Franks DW, James R (2011) Hypothesis testing in animal social networks. Trends Ecol Evol 26:502–507

Drewe JA (2009) Who infects whom? Social networks and tuberculosis transmission in wild meerkats. Proc R Soc Lond B 277:633–642

Fenner AL, Godfrey SS, Bull CM (2011) Using social networks to deduce whether residents or dispersers spread parasites in a lizard population. J Anim Ecol 80:835–843

Ferrari N, Cattadori IM, Nespereira J, Rizzoli A, Hudson PJ (2003) The role of host sex in parasite dynamics: field experiments on the yellow-necked mouse *Apodemus flavicollis*. Ecol Lett 6:1–7

Godfrey SS, Bull CM, James R, Murray K (2009) Network structure and parasite transmission in a group living lizard, gidgee skink, *Egernia stokesii*. Behav Ecol Sociobiol 63:1045–1056

Godfrey SS, Moore JA, Nelson NJ, Bull CM (2010) Social network structure and parasite infection patterns in a territorial reptile, the tuatara (*Sphenodon punctatus*). Int J Parasitol 40:1575–1585

Grear DA, Perkins SE, Hudson PJ (2009) Does elevated testosterone result in increased exposure and transmission of parasites? Ecol Lett 12:528–537

Hawley DM, Etienne RS, Ezenwa VO, Jolles AE (2011) Does animal behavior underlie covariation between hosts' exposure to infectious agents and susceptibility to infection? Implications for disease dynamics. Integr Comp Biol 5:528–539

Holekamp K (1984) Dispersal in ground-dwelling Sciurids. In: Murie JO, Michener GR (eds) The Biology of Ground-dwelling Squirrels. University of Nebraska, Lincoln, pp 295–320

Hou L, Li X, Moeller R, Palermo B, Atwill ER (2004) Neonatal-mouse infectivity of intact *Cryptosporidium parvum* oocysts isolated after optimized in vitro excystation. Appl Environ Microbiol 70:642–646

Keeling MJ (1999) The effects of local spatial structure on epidemiological invasions. Proc R Soc Lond B 266:859–867

Keeling MJ (2005) The implications of network structure for epidemic dynamics. Theor Popul Biol 67:1–8

Keeling MJ, Eames KTD (2005) Networks and epidemic models. J R Soc Interface 2:295–307

Lloyd-Smith JO, Schreiber SJ, Kopp PE, Getz WM (2005) Superspreading and the effect of individual variation on disease emergence. Nature 438:355–359

McCallum H, Barlow N, Hone J (2001) How should pathogen transmission be modelled? Trends Ecol Evol 16:295–300

McLean IG (1984) Spacing behavior and aggression in female ground squirrels. In: Murie JO, Michener GR (eds) The biology of ground-dwelling squirrels. University of Nebraska, Lincoln, pp 321–335

Michener GR (1984) Age, sex, and species differences in the annual cycles of ground-dwelling Sciurids: implications for sociality. In:

Murie JO, Michener GR (eds) The biology of ground-dwelling squirrels. University of Nebraska, Lincoln, pp 79–107

Murie JO, Michener GR (1984) Biology of ground-dwelling squirrels. University of Nebraska, Lincoln

Newman MEJ (2003) Properties of highly clustered networks. Phys Rev E 68:1–6

Nunes S, Muecke EM, Anthony JA, Batterbee AS (1999) Endocrine and energetic mediation of play behavior in free-living Belding's ground squirrels. Horm Behav 36:153–165

Otterstatter MC, Thomson JD (2007) Contact networks and transmission of an intestinal pathogen in bumble bee (*Bombus impatiens*) colonies. Oecologia 154:411–421

Perkins SE, Ferrari MF, Hudson PJ (2008) The effects of social structure and sex-biased transmission on macroparasite infection. Parasitology 135:1561–1569

Perkins SE, Cagnacci F, Stradiotto A, Arnoldi D, Hudson PJ (2009) Comparison of social networks derived from ecological data: implications for inferring infectious disease dynamics. J Anim Ecol 78:1015–1022

Porphyre T, McKenzie J, Stevenson MA (2011) Contact patterns as a risk factor for bovine tuberculosis infection in a free-living adult brushtail possum *Trichosurus vulpecula* population. Prev Vet Med 100:221–230

Turner J, Bowers JA, Clancy D, Behnke MC, Christley RM (2008) A network model of *E coli* O157 transmission within a typical UK dairy herd: the effect of heterogeneity and clustering on the prevalence of infection. J Theor Biol 254:45–54

Valente TW (2010) Social networks and health: models, methods, and applications. Oxford University, Oxford

Wasserman S, Faust K (1994) Social network analysis: methods and applications. Cambridge University, Cambridge

Wu X, Liu Z (2008) How community structure influences epidemic spread in social networks. Physica A 387:623–630

Zu S-X, Fang G-D, Fayer R, Guerrant RL (1992) Cryptosporidiosis: pathogenesis and immunology. Parasitol Today 8:24–27