Primate reinfection with gastrointestinal parasites: behavioural and physiological predictors of parasite acquisition

Sagan Friant ^{a,b}, Toni E. Ziegler ^c, Tony L. Goldberg ^{*a,b,d}

^a Nelson Institute for Environmental Studies, University of Wisconsin-Madison

^b Wisconsin National Primate Research Center, Madison

^c Global Health Institute, University of Wisconsin-Madison

^d Department of Pathobiological Sciences, School of Veterinary Medicine, University of Wisconsin-Madison

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***Author for correspondence:**

Tony L. Goldberg

Email: tgoldberg@vetmed.wisc.edu

University of Wisconsin-Madison

Hanson Laboratories, room 137

1656 Linden Drive

Madison, Wisconsin 53706

Phone: 608-890-2618 (office); 608-890-3255 (lab)

FAX: 608-262-7420

In humans and other social animals, variation in behaviour and physiology can alter the risk of exposure to, and infection by, pathogens, ultimately affecting host fitness (Kappeler, Cremer, & Nunn, 2015; Nunn, Craft, Gillespie, Schaller, & Kappeler, 2015; Silk, 2014). Variation in parasitism often has direct links to host social behaviour, such that infection-related costs of sociality are considered important selective forces in human and animal evolution (Altizer et al., 2003; Kappeler et al., 2015; Møller, Dufval, & Allander, 1993). Clarifying the mechanisms whereby sociality translates to infection is important for our understanding of disease ecology and host-parasite co-evolution. For example, it is currently unclear whether close proximity and high levels of contact or increased physiological stress resulting from within-group social dynamics is more important for infection in primates.

Macroparasites are generally aggregated within populations, with few hosts harbouring the majority of infections (Crofton, 1971; Poulin, 2007; Shaw & Dobson, 1995). Classic measures typically associated with infection include age, sex, and dominance status (Nunn & Altizer, 2006). Behavioural and physiological mechanisms that influence encounter rates and immune status can vary with these measures, further explaining why certain individuals are at increased risk of infection. Focus on classic measures alone may therefore obscure important contributions of social connectivity and/or physiological stress (Cavigelli & Caruso, 2015; Kappeler et al., 2015).

In primates, trade-offs between sociality, encounter rates, and immune-function result in conflicting predictions for disease risk of individuals (Nunn & Altizer, 2006). For example, age can increase parasitism if larger bodied individuals occupy more space, require more resources, and contact contaminated foods and substrates disproportionately. Conversely, lack of acquired immunity in younger individuals may increase risk of parasitism in juveniles (Hudson & Dobson, 1997). Parasitism tends to be more common in males than in females across vertebrate taxa (Habig &

Archie, 2015). However, male-biased parasitism is confounded by body size, such that the immunosuppressive effects of stress and testosterone are unclear (Zuk & McKean, 1996). In primate societies with dominance hierarchies, greater access to resources and rank-mediated social contact should increase risk for high-ranking individuals (MacIntosh et al., 2012; Rushmore et al., 2013). Meanwhile, immunosuppressive effects of stress hormones can increase susceptibility in either dominant or subordinate individuals depending on species-typical dynamics and hierarchical stability (Cavigelli & Caruso, 2015; Sapolsky, 2005).

Empirically, intraspecific differences in physical contact, proximity (González-Hernández et al., 2014; MacIntosh et al., 2012; Rimbach et al., 2015), and physiological stress (Chapman, Saj, & Snaith, 2007; Clough, Heistermann, & Kappeler, 2010; Muchlenbein, 2006) are associated with transmission of parasites within primate groups. In Japanese macaques (*Macaca fuscata*), for example, socially mediated exposure seems to be more important than the immunosuppressive effects of stress in explaining why dominant females have more infections from directly transmitted parasites (MacIntosh et al., 2012). Nevertheless, the relative importance of network connectivity versus physiological stress as mechanisms for facilitating pathogen spread is not well understood.

In this study, we investigated how social connectivity and physiological stress compare to host intrinsic factors with respect to explaining patterns of parasite aggregation in primates. To overcome confounding heterogeneities in exposure, susceptibility, and resulting infection levels over time, we experimentally removed parasites and measured rate to reinfection. To date, experimental manipulations of parasite infections in wild animals have focused primarily on behavioural, immune, and fitness responses to parasitism (Coster, Neve, Martín-Gálvez, Therry, & Lens, 2010; Hillegass, Waterman, & Roth, 2010; Raveh, Neuhaus, & Dobson, 2015). Here, we investigated patterns of parasite reacquisition following chemotherapeutic treatment of red-capped

mangabeys (*Cercocebus torquatus*) for gastrointestinal helminth and protozoan parasites.

Specifically, we examined how centrality within social networks and individual stress varied within the population according to sex, age, and dominance. We then compared how these mechanistic explanations (e.g. contact, proximity and/or stress) performed against classic measures in predicting rate to reinfection. We compared reinfection from helminths and protozoans separately, given their inherent differences in time-to-infection and aggregation within hosts (Shaw & Dobson, 1995). By focusing on gastrointestinal parasites, which can be collected non-invasively and can be treated with oral medications, we are able to compare results from our field experiment to other observational studies that also investigated gastrointestinal parasites. We predicted that following experimental manipulation of infection, centrality would augment classic measures to more powerfully explain differences in infection rates.

METHODS

Study Site and Population

The study took place at Rhoko Research and Conservation Education Centre (41.21° N, 16.16° E), the forest site of the Centre for Education, Research and Conservation of Primates and Nature (CERCOPAN). Rhoko is located in the transition zone surrounding the Oban division of Cross River National Park in Cross River State, Nigeria. The vegetation is characteristic of lowland rainforest, forming a mosaic of disturbed and relatively undisturbed forest patches. Climate includes a long wet season from April to November and a short dry season from November to March.

We studied 49 red-capped mangabeys living in a multi-male, multi-female social group, all of which had been rescued from the bushmeat and pet trades as young juveniles or were first- to third-generation captive born. The group was housed in a one-hectare open-topped forest enclosure with full canopy cover and within the natural home range of the species. The population was provisioned

daily, but also ate wild foods opportunistically and drank from a stream running through the enclosure. Animals were vulnerable to natural predators (e.g. snakes and birds of prey) and parasites. All animals were well habituated and individually recognizable to the trained observer through individual differences in size, pelage, and facial characteristics; all data were collected from animals where observers had achieved 100% agreement on identification. The age of each individual at the start of data collection (range: 1.08-18.5) was known from birth records or estimated from tooth wear, pelage characteristics and sexual maturity at date of rescue. We categorized males as adult (≥ 6 yr, $n=9$), sub adult (≥ 3 yr, $n=9$), and juvenile (< 3 yr, $n=7$), and females as adult (≥ 4 yr, $n=19$) or juvenile (< 4 yr, $n=5$). *Cercocebus torquatus* is currently listed as vulnerable by IUCN (Oates, Gippoliti, & Groves, 2008).

Chemotherapeutic Treatment

In June of 2012, the entire population was treated for gastrointestinal parasites via simultaneous administration of metronidazole (50 mg/kg for 7 days) for protozoans, mebendazole (50 mg/kg for 3 days) for nematodes, and praziquantel (20mg/kilo for 3 days) for cestodes and trematodes. For all drugs, a single dose was delivered in maize cereal to identified individuals to ensure that each animal received at least one dose. The remaining doses were dissolved in fruit and administered via group feeds following standard practice for population level treatment at CERCOPAN. The treatment period lasted ten days in total.

Study Design

The study took advantage of a planned treatment event, providing a unique opportunity to measure patterns of infection following an intervention and minimizing additional risk. The treatment regimen was developed from standard treatment practices at CERCOPAN and in consultation with two wildlife veterinarians. Oral administration of drugs and non-invasive

assessment of parasites were used to minimize adverse risks and enhance welfare. The Institutional Animal Care and Use Committee at University of Wisconsin-Madison approved all research activities (protocol V1490).

Faecal samples and behavioural and health data were collected between May and September 2012. Faecal sampling was conducted one month prior to treatment and paralleled behavioural sampling for three months immediately following treatment. Pre-treatment faecal samples were collected in triplicate from each individual to increase detection of parasites shed intermittently. To assess variation in protozoan infection, which are infectious upon shedding and have short pre-patent periods, post-treatment faecal samples were collected at the highest frequency for the month immediately following treatment (approx. every three days/ individual). Time to infection from protozoans was measured during the period of high sampling intensity only (30 days post-treatment). Sampling intensity was gradually decreased in the second month (approx. every five days) and third month (approx. every 10 days) to detect new infections from helminths, which develop in the external environment and have longer pre-patent periods, such that helminth reinfection was measured over this longer time period (80 days post treatment). Hormones were extracted from triplicate samples directly following treatment (approx. every 10 days), with sampling intensity reduced to twice a month (approx. every 15 days) for the second and third months.

Behavioural Data Collection

Associational data were collected by recording all grooming partners, direction of grooming, and nearest neighbours (within 2 m) of focal individuals during three observation periods daily: early morning (7:00-10:00), mid-morning (11:00-15:00), and evening (16:00-19:00). The mangabeys were often dispersed in dense undergrowth throughout their enclosure,

making it difficult to collect data on a predetermined schedule or in a specific order. Individuals were therefore selected opportunistically. A minimum of 1.5 minutes was allowed to elapse between observations to reduce interdependence of data. An individual was not sampled if it was an associate or nearest neighbour in the preceding observation. Dominance data was collected by recording all observed agonistic interactions and directionality of submissions and supplants using structured *ad libitum* sampling, as conducted for similar species (Range & Noe, 2002). All data were collected by three observers for 81 days over 3 months post-treatment. Inter-observer reliability was tested and accepted by calculating Fleiss's Kappa test for categorical agreements between multiple observers (Kappa= 0.89, $p < .001$ for observers 1 and 2; Kappa = 0.92, $p < .001$ for observers 1 and 3).

Faecal collection and preservation

Faecal samples were collected immediately following defecation and fixed within two hours of collection. Separate aliquots of each sample were used for preservation of gastrointestinal parasites and hormones. First, a 2 g aliquot was taken from within the faecal mass and stored in a 3:1 ratio of 10% formalin to faeces for preservation of gastrointestinal parasites (Greiner & McIntosh, 2009). A second aliquot was then separated, mixed thoroughly, and 0.50 - 0.55 g weighed into a 15 ml tube. Equal parts (2.5 ml) distilled water and ethanol were mixed with the sample by shaking vigorously for five minutes for preservation of hormones (Ziegler & Wittwer, 2005).

Hormones were extracted in the field via solid phase extraction (SPE) (Ziegler & Wittwer, 2005). Samples were stored for over 24 hours until faecal material naturally sedimented at the bottom of the tube and a clear supernatant was evident. Supernatant was decanted into a small weighing dish, and 2 ml was removed and pushed through SPE columns (Prevail C18,

Alltech, Deerfield, IL) using a 5 ml disposable syringe at a flowrate of 1 ml/min. Solid phase extraction columns were then washed by pushing 1 ml of distilled water through cartridges at the same rate to eliminate contaminants. Cartridges were capped and stored at room temperature out of direct sunlight until transport. Formalin preserved samples and SPE cartridges were transported to the University of Wisconsin, Madison following all applicable import, export, and International Air Transport Association regulations.

Behavioural data analysis

Dominance matrices were constructed from post-treatment dyadic supplants and aggressive and avoidance interactions between adults of the same sex using SOCPROG 2.6 (Whitehead, 2009). David's scores (DS) were used to dichotomize individuals as 'usually dominant' ($DS > 0$) or 'usually subordinate' ($DS < 0$) (de Vries, Stevens, & Vervaecke, 2006). Linearity of male and female hierarchies was assessed using de Vries' test and h' with 1000 permutations for dominance hierarchies containing unknown or tied relationships (de Vries et al., 2006).

Adjacency matrices for social network analyses were constructed directly from observed post-treatment pairwise associations ("proximity" network) and directional grooming interactions ("contact" networks) using SOCPROG 2.6 (Whitehead, 2009). Matrices with attribute information were imported into R version 3.2.2 (R Core Team, 2014), where all further analyses were conducted unless otherwise specified. Calculations of symmetric network metrics from proximity matrices, and asymmetric metrics from directional contact matrices were performed using the *sna* package (Farine & Whitehead, 2015). Node-based measures commonly used for modelling transmission of infectious diseases were calculated for each individual in each network: 1) degree centrality (hereafter referred to as centrality) – number of associates or

interactants, 2) strength – number of associations or interactions, 3) closeness – shortest number of paths needed to reach all other individuals, and 4) betweenness – number of shortest paths going through an individual (Drewe & Perkins, 2015). Indegree and outdegree were calculated for directional networks, representing groom-receive and groom-give respectively.

Given the non-independence of data in network analyses, network metrics were compared to host characteristics using permutation tests. Null models were built from data stream based randomizations and significance was measured by comparing the test statistic of the models fitted to the observed data with the test statistic calculated from 1000 permutations of the network using the *asnipe* package (Farine, 2013; Farine & Whitehead, 2015). Cohen's d effect size was calculated for all comparisons using the *compute.es* package (Farine & Whitehead, 2015; Re, 2015). Network diagrams were constructed using UCINET software's NetDraw program (Borgatti, Everett, & Freeman, 2002), with node size representing individual centrality, weighted edges representing strength, and without filters.

Parasitology

One gram of formalin preserved faeces was concentrated via faecal sedimentation following the protocol of Greiner and McIntosh 2009 for assessment of gastrointestinal parasites of primates (Greiner & McIntosh, 2009). Briefly, 1 g of faeces was suspended in 40 ml of sedimentation solution (soapy water), mixed gently to avoid formation of bubbles, and filtered twice through a cheesecloth to remove large debris. The mixture was left to sediment for 10 minutes, after which the supernatant was decanted. The remaining pellet was re-suspended in distilled water and left to sediment for another 10 minutes. Supernatant was then removed with a transfer pipette, and sediment was preserved in formalin until examination. The entirety of the sediment was systematically examined at X10 objective light magnification for helminth eggs

and larvae. One drop of sediment from each sample was then examined at X40 for identification of protozoan cysts. Representative parasites were measured with a calibrated ocular micrometer and photographed at X40 magnification. All helminth eggs, larvae, and protozoan cysts were assigned to taxa based on their size, shape, colour, and contents. Parasite richness (number of different parasite taxa within a host) and prevalence (percent of individuals infected with a particular parasite taxon) were calculated (Bush, Lafferty, Lotz, & Shostak, 1997). Efficacy of treatment was evaluated by examining changes in parasite richness over time (before and after treatment) using one-way analyses of variance (ANOVA) and Tukey HSD post-hoc test. Prior to this study, parasite communities had not been reported for this species.

Faecal cortisol analysis

Faecal cortisol levels were measured via enzyme immunoassay at the Wisconsin National Primate Research Center Assay Services Unit. Steroid hormones were eluted using 2 ml of 100% methanol after washing cartridges with 1 ml 5% methanol. Eluted samples were then evaporated and hormones were rehydrated in 1 ml of 100% ethanol and stored at 4°C. Prior to EIA 25 µl was removed from each sample and evaporated. Assays were performed using R4866 (anti-cortisol-bovine serum albumin) developed by Stabenfeldt and Munro at the University of California, Davis, with 60% cross-reactivity to corticosterone (previously described in (Ziegler, Scheffler, & Snowden, 1995)). Plates were read using SpectraMax 340PC microplate reader. Recovery was 105.68% ± 3.15%. Parallelism was demonstrated using serial dilution curves derived from high value faecal extracts with no significant difference from the slope of the standard curve ($t=.93$, $df=24$, $P>.05$). Interassay variation for the high pool was 18.3% and for the low pool was 22.2%, whereas intra-assay variation was 3.8% for the high pool and 7.9% for the low pool. Average post-treatment faecal cortisol levels (ng/g) were compared across sex, age,

and dominance status using Spearman rank correlations and Mann-Whitney U tests. Prior to this study, cortisol had not been reported for this species.

Statistical Analysis

Marginal cox proportional hazards models for multiple events data were used to examine host traits, behaviour, and physiology as predictors of time to reinfection (Kleinbaum & Klein, 2012; Wei, Lin, & Weissfeld, 1989). The marginal approach focuses on the total time from study entry to occurrence of each event, thereby combining time to infection and number of infections (*i.e.* richness) within a single model. In these models, the baseline hazard function (*i.e.* dependent variable) was defined as time to infection, and stratified by parasite type. Stratification by parasite within models allowed the baseline hazard function to vary for each parasite taxon. New infections were defined as shedding of cysts/eggs in the post-treatment sampling period after testing negative for the entirety of the parasite-specific pre-patent period. Because drug administration typically reduces burden, but does not typically clear infection entirely (Pedersen & Fenton, 2015), we included only parasites that showed more than 50% reduction in prevalence and contributed to subsequent reinfections. Individuals that did not experience an event by the end of the study were right-censored (e.g. time to infection for these individuals was considered to be at least as long as the duration of the study).

Individual characteristics including sex, age class, dominance status, faecal cortisol levels, and centrality measures were incorporated as covariates in maximal models. Robust variance estimates were assigned to adjust for the likely correlation among multiple events on the same subject (Lin & Wei, 1989). To control for the increased opportunity for infection in individuals successfully treated for all parasites (compared to those that retained some infections

following treatment), we forced a covariate into each model to represent the maximum number of events possible per subject.

Separate maximal models were built for protozoan and helminth infections. Because juveniles and sub-adults were not assigned dominance values, we ran two models under each category: a) all ages (dominance excluded), and b) adults only (dominance included). Centrality measures were incorporated into maximal models one at a time (i.e. each model only contained one centrality metric from one network) to avoid multicollinearity. Model selection was then carried out independently for each model using the likelihood ratio test (LRT) selection criterion (Kleinbaum & Klein, 2012). Backwards elimination of predictor variables was used to select models that retained only significant covariates (at the $\alpha < .05$ level) and first order interactions. Permutation-based methods, which are often used in the statistical analysis of network data (Croft, Madden, Franks, & James, 2011), were not utilized here because the response variable was not based on relational data (VanderWaal, Atwill, Hooper, Buckle, & McCowan, 2013). Models were accepted if they had generalized variance inflation factors (GVIF) within reason (< 4) (O'brien, 2007), satisfied the proportional hazards assumption (residuals were not significantly correlated with time [ZPH: Pearson's ρ , $P > 0.05$]), and explained significantly more variance than the null model (Likelihood-ratio test [LRT], $P < 0.05$) (Kleinbaum & Klein, 2012)

Hazard ratios (HR), the ratio of the chance of parasite acquisition in one level of an explanatory variable relative to the other, were reported for all significant predictors. Survival curves were produced for significant predictors using the Kaplan-Meier method. Centrality scores were dichotomized at the median because hazard ratios and Kaplan-Meier curves are more interpretable when comparing groups, and because in general, models with dichotomized

variables out performed those with continuous variables (i.e. produced higher log likelihoods and fewer ZPH violations). We performed all analyses with the *surv*, *coxph*, and *cox.zph* functions in the *survival* package using R version 3.2.2 (R Core Team, 2014; Therneau, 2015).

RESULTS

Dominance

Dominance matrices were constructed from 628 female ($\mu + SD = 66.11 + 30.94$ per individual; $\mu + SD = 3.48 + 1.63$ per dyad) and 368 male ($\mu + SD = 81.78 + 32.33$ per individual, $\mu + SD = 9 + 3.59$ per dyad) dyadic dominance interactions. For both male and female hierarchies, deVries test of linearity indicated that dominance was moderately linear and non-random for female ($h' = 0.73$, $p < 0.001$) and male ($h' = 0.84$, $p < 0.01$) hierarchies.

Centrality

Proximity networks were constructed from 2,374 observed pairwise associations ($\mu + SD = 96.50 + 48.54$ per individual; $\mu + SD = 1.97 + 0.98$ per dyad) and contact networks from 555 dyadic grooming interactions ($\mu + SD = 11.32 + 9.37$ per individual; $\mu + SD = 0.23 + 0.19$ per dyad). Directed contact networks were more heterogeneous (varied across sex, age, and dominance status) compared to proximity networks (Fig. 1). Females had higher closeness ($t_{47}=4.64$, $P < 0.001$, $d=1.33$), and betweenness ($t_{47}=3.64$, $P < 0.001$, $d=1.04$), in the contact networks compared to males, and higher centrality ($t_{47}=5.83$, $P < 0.001$, $d=1.66$) and strength ($t_{47}=6.17$, $P < 0.05$, $d=1.76$) measures in the groom-give network. Adults had significantly higher closeness ($t_{47}=3.63$, $P < 0.001$, $d=1.05$) in the contact networks; and higher centrality ($t_{47}=6.15$, $P < 0.001$, $d=1.78$) and strength ($t_{47}=4.08$, $P < 0.05$, $d=1.18$) in groom-receive network. High-ranking males were more central in the groom-give network ($t_7=2.97$, $P < 0.001$, $d=1.99$), and had stronger connections in both proximity ($t_7=2.19$, $P < 0.05$, $d=1.47$) and groom-receive

networks ($t_7=2.61$, $P < 0.01$, $d=1.75$). Dominant females had higher closeness ($t_{17}=2.58$, $P < 0.01$, $d=1.2$) and betweenness ($t_{17}=2.02$, $P < 0.001$, $d=0.94$) in contact networks, and higher centrality ($t_{17}=2.14$, $P < 0.01$, $d=0.99$) and stronger connections in groom-give network ($t_{17}=3.05$, $P < 0.001$, $d=1.42$).

Faecal cortisol

A total of 343 post-treatment faecal samples (7 per individual) were collected for hormone analyses. Faecal cortisol levels ranged from 0.57 ng/g to 49.39 ng/g among individuals. Cortisol levels were positively related to age ($r_s=.55$, $P < 0.001$), and low-ranking individuals had higher levels ($M=12.77$ ng/g) than high-ranking individuals ($M=8.77$ ng/g) ($U=50$, $P < 0.05$). Females ($M=9.95$ ng/g) had marginally higher cortisol values compared to males ($M=8.07$ ng/g) ($U=215$, $P < 0.1$). There was no relationship between centrality within social networks and average faecal cortisol levels.

Parasitism

A total of 982 faecal samples were collected, for an average of 20 samples per individual. Mangabeys were infected with six protozoan and nine helminths prior to treatment (Table 1). Average protozoan ($F_{5, 287} = 39.22$, $P < 0.01$) and helminth ($F_{7, 383} = 13.18$, $P < 0.01$) richness differed significantly over time. Post-hoc comparisons showed significant reductions in parasite richness between pre-treatment (helminth $\mu + SD = 1.55 + 1.29$, $P < 0.001$; protozoa $\mu + SD = 2.77 + 1.36$, $P < 0.001$) and post-treatment samples (helminth $\mu + SD = .67 + .87$; protozoa $\mu + SD = 0.27 + 0.86$). Protozoan infections were more quickly acquired than helminth infections. There was no significant difference between pre-treatment richness and richness at the final sample for helminths or protozoans, demonstrating return to baseline levels (Fig. 2a,b). Prevalence of five protozoans and four helminths were reduced by at least 50% and contributed to

subsequent reinfections (Table 1). These nine parasites were therefore included in cox proportional hazard models. Protozoans and helminths showed different distributions at the final time points, further supporting our decision to model infection separately for each group of parasites (Fig. 2c,d). A right skewed distribution of helminth richness showed that a small number of individuals harboured many parasite taxa whereas most individuals were infected with only a few taxa (Fig. 2d).

Following treatment (and prior to the end of the study), 81 new helminth infections (48 in adults) and 189 new protozoan infections (111 in adults) were detected. High centrality, closeness, and betweenness in proximity networks were associated with rate to infection with helminth parasites in adult mangabeys (Fig. 3; Table 2). When all age classes were included, these trends were in the same direction, but were marginal. No centrality measures explained time to reinfection with protozoans. Neither host traits (e.g. sex, age, or dominance) nor average post-treatment cortisol levels were associated with rate to infection under either model.

DISCUSSION

Measures of social centrality were more important than individual host traits (e.g. sex, age, and dominance) or physiological stress for explaining rates of infection with helminth parasites in mangabeys. Specifically, we found that individuals with a higher number of associates (centrality), that were well connected (closeness), and that had a tendency to switch groups (betweenness), were at higher risk of acquiring helminth parasites. Number of associations (strength), and measures of connectivity within contact networks were not associated with infection risk. Acquisition of protozoan infections, which do not tend to aggregate in populations, did not vary according to centrality, stress, or host traits. Together, our results show that parasite aggregation was determined primarily by host associations, and that

social connectivity, rather than the immunosuppressive effect of stress, may explain enhanced infection risk.

The probability of acquiring new helminth infections was higher in animals that were central in the proximity network. This finding demonstrates a potential cost associated with social connectivity. A growing body of evidence suggest that social centrality increases risk of infection from macroparasites. In primates, centrality within grooming networks has been indirectly associated with nematode infections in female Japanese macaques (*Macaca fuscata yuki*), and directly associated with parasite species richness in brown spider monkeys (*Ateles hybridus*), (MacIntosh et al., 2012; Rimbach et al., 2015). Our findings differ from studies of brown spider monkeys, however, in which centrality in contact networks was more important than proximity for explaining elevated infection risk (Rimbach et al., 2015).

Helminths must develop in the external environment for days to months before they become infective. Risk associated with connectivity in proximity networks, rather than contact networks, may therefore reflect increased exposure to infectious life stages persisting on fomites and food items (Freeland, 1980). We cannot rule out the possibility that spatial associations, serve as proxy for contact (Farine, 2015). However, this is unlikely given that grooming networks showed a more heterogeneous structure (Fig. 1), despite being based on fewer observations. Indeed, space sharing has been identified as an important predictor of risk for infection from macroparasites in other taxonomic groups (Fenner, Godfrey, & Bull, 2011; Godfrey, Moore, Nelson, & Bull, 2010; Perkins, Cagnacci, Stradiotto, Arnoldi, & Hudson, 2009).

We found that protozoans, which are immediately infective once shed into the environment, were acquired uniformly across the population. This result aligns with our current understanding of the transmission biology of protozoan parasites, which tend not to aggregate in

hosts like macroparasites (Shaw & Dobson, 1995)(Fig. 2). Similarly, centrality was associated with parasite richness in brown spider monkeys, but these results were not significant for a protozoan (*Entamoeba* spp.) alone (Rimbach et al., 2015). In other taxa, however, contact appears to be important for predicting the spread of directly transmitted and immediately infectious pathogens such as *Mycobacterium bovis* in meerkats (*Suricata suricatta*) (Drewe, 2009), and fungi in garden ant (*Lasius neglectus*) colonies (Theis, Ugelvig, Marr, & Cremer, 2015).

Unfortunately, low prevalence often reduces the power of transmission mode-specific models such as the ones we constructed. Nevertheless, systematic studies using aggregate models are important, since cumulative effects of multiple infections can have marked impacts on host health and fitness (Bordes & Morand, 2011). Identifying the hosts with high rates of infection from multiple parasites can therefore help identify fitness costs associated with social behaviour. Such models will be critical in improving our understanding of the role of social contact in pathogen spread (Craft, 2015; Gear, Luong, & Hudson, 2013).

We did not find an effect of glucocorticoid level on acquisition of parasites. Similarly, in female Japanese macaques, social contact was determined to be more important than the immunosuppressive effects of stress in mediating the relationship between dominance and parasite infection (MacIntosh et al., 2012). Parasitism in white-handed gibbons (*Hylobates lar*) and black howler monkeys (*Alouatta pigra*) was also not affected by faecal cortisol (Gillespie, Barelli, & Heistermann, 2013; Martinez-Mota, 2015); although, positive associations between parasitism and faecal cortisol have been documented in other primate species (Arlet et al., 2015; Foerster, Kithome, Cords, & Monfort, 2015; Muehlenbein, 2006). The lack of predictable outcomes may be

due, in part, to the dynamics of the stress response (Cavigelli & Caruso, 2015), and competing effects of acute and chronic cortisol elevations on immunity (Dhabhar & McEwen, 1999).

Our data show that the nature of connections within a network impact infection risk. In our study, dominant males had stronger relationships in proximity networks, and females, dominant animals, and adults tended to have higher connectivity in grooming networks. However, none of these centrality measures were associated with parasite acquisition. Attempts to link transmission of pathogens to certain host traits based on centrality metrics alone should therefore be interpreted with caution. Furthermore, risk associated with social connectivity is not likely to be static, but rather can vary over time (Rushmore et al., 2013). Heterogeneities in social connectivity, and resulting changes in infection over time, may therefore obscure the relationship between social network position and individual infection risk in cross-sectional studies.

Conclusions

In our study population of Nigerian red-capped mangabeys, animals central in the social network had a higher probability of acquiring macroparasites than did peripheral individuals. Individuals with high centrality may facilitate transmission throughout the population, perhaps acting as “super spreaders” (Lloyd-Smith, Schreiber, Kopp, & Getz, 2005). We found no direct associations between intrinsic host traits and time to infection, which concords with the result of previous studies. In fact, individual predictors of parasite aggregation (e.g. sex, age, and dominance) documented in previous studies may be confounders of the direct relationship between social connectivity and infection risk. Overall, our data suggest that being central in a social network confers costs in terms of infection risk. Variation in social networks structure and dynamics should be considered in studies of infection risk in social species.

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Table 1. Biological classifications of parasites recovered from faeces of red-capped mangabeys showing prevalence prior to and immediately following de-worming, and at the final time point for protozoa and helminths.

Classification	Genus	Species	Life Cycle	Mode of Transmission	Prevalence ^a		
					pre-	post-	final
Protozoa ^b	<i>Entamoeba</i> *	<i>hartmanni</i>	direct	Ingestion of trophozoites and cysts	.80	.02	.51
	<i>Entamoeba</i> *	<i>histolytica/ dispar</i>	direct	“	.98	.02	.78
	<i>Entamoeba</i> *	<i>coli</i> +	direct	“	.69	.02	.67
	<i>Iodamoeba</i> *	<i>bütschlii</i>	direct	“	.69	.02	.61
	<i>Chilomastix</i> *	<i>mesnili</i>	direct	“	.47	.00	.55
	<i>Balantidium</i>	<i>coli</i>	direct	“	.88	.02	0
	<i>Trichuris</i>	<i>trichiura</i>	direct	Ingestion of eggs	.02	0	0
Nematoda	<i>Capillaria</i>	sp.	unknown	unknown	.06	0	.04
	<i>Strongyloides</i> *	sp.	direct	Ingestion of eggs/ penetration of skin by larvae	.22	.06	.35
	<i>Enterobius</i>	sp.	direct	Ingestion of eggs	.16	.02	0
	Hookworm*		direct	Penetration of skin by larvae	.12	.02	.12
	unknown*	sp.	Unknown	unknown	.06	0	.12
	<i>Abbreviata</i> *	sp.	indirect1	Ingestion of arthropod	.87	.28	.59
	<i>Paragonimus</i>	<i>africanus</i>	indirect2	Ingestion of crab	.43	.35	.43
Cestoda	<i>Bertiella</i>	sp.	indirect1	Ingestion of mite	.04	0	0

* Indicates parasite met criteria for incorporation into cox proportional hazards models

^a Prevalence calculated from triplicate samples collected prior to treatment (pre), single post treatment sample (protozoan; n=45), and triplicate samples collected at the end of the study

^b Species identifications putative based on size and morphological characteristics of cysts

Table 2. Marginal Cox proportional hazard models associating host characteristics to rate to infection with parasites.

Response variable	Network type	Predictors ^{a,b}	p> z	HR (95% CI)			LRT	ZPH	GVIF ^c
Helminth reinfection	proximity	Degree	<.01	2.33	1.39	3.91	<.01	.87	< 4
		Strength	ns						
		Betweenness	<.05	1.91	1.02	3.57	<.05	.96	< 4
		Closeness	<.01	2.20	1.33	3.63	<.01	.94	< 4
	contact^d		ns						
Protozoan reinfection	proximity		ns						
	contact		ns						

^a Sex, age, dominance status and faecal cortisol were not significant under any model

^b Reference level is central.

^c Generalized variance inflation factors met the rule GVIF < 4 for all maximal models.

^d In degree/out degree (groom-receive) and in strength/out strength (groom-give) were incorporated into models as measures of centrality within contact networks.

Figure 1. Mangabey social networks showing position of individuals according to host traits.

Nodes represent individuals, and edges (line connecting nodes) represent proximity (a), and directed grooming [groom- receive (b) and groom-give (c)]. Node size and colour represent host characteristics, including: sex (female=red; males=blue), age (juveniles and sub-adults=triangles) and dominance status (high-ranking=circle; low-ranking=square). Individuals with more contacts (high degree centrality) are represented by larger nodes. Directed relationships are indicated by edges with arrows. Thickness of lines represents strength of the relationship (strength). No filters were applied.

Figure 2. Average parasite richness at each sample point relative to treatment (*dashed red line*) for protozoans (a) and helminths (b). Treatment led to a significant reduction in both protozoan and helminth richness, and richness returned to baseline levels for both parasite groups by the end of the study. Error bars show standard error of the mean. Histograms show different distributions of protozoan (c) and helminth (d) parasites within the host population at censoring.

Figure 3. Kaplan-Meier curves showing rate to reinfection following treatment for parasites. Individuals with high degree and closeness centrality (a) and betweenness (b) in proximity networks became infected with helminth parasites more quickly than did peripheral individuals.