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Social buffering *and* contact transmission: Network connections have beneficial and detrimental effects on *Shigella* infection risk among captive rhesus macaques

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In social animals, group living may impact the risk of infectious disease acquisition in two ways. On the one hand, social connectedness puts individuals at greater risk or susceptibility for acquiring enteric pathogens via contact-mediated transmission. Yet conversely, in strongly bonded societies like humans and some nonhuman primates, having close connections and strong social ties of support can also socially buffer individuals against susceptibility or transmissibility of infectious agents. Using social network analyses, we assessed the potentially competing roles of contact-mediated transmission and social buffering on the risk of infection from an enteric bacterial pathogen (*Shigella flexneri*) among captive groups of rhesus macaques (*Macaca mulatta*). Our results indicate that, within two macaque groups, individuals possessing more direct and especially indirect connections in their grooming and huddling social networks were *less susceptible* to infection. These results are in sharp contrast to several previous studies that indicate that increased (direct) contact-mediated transmission facilitates infectious disease transmission, including our own findings in a third macaque group in which individuals central in their huddling network and/or initiated more fights were *more likely* to be infected. In summary, our findings reveal that an individual's social connections may increase *or* decrease its chances of acquiring infectious agents. They extend the applicability of the social buffering hypothesis, beyond just stress and immune-function-related health benefits, to the additional health outcome of infectious disease resistance. Finally, we speculate that the circumstances under which social buffering versus contact-mediated transmission may occur could depend on multiple factors, such as living condition, pathogen-specific transmission routes, and/or an overall social context such as a group's social stability.

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Abstract

In social animals, group living may impact the risk of infectious disease acquisition in two ways. On the one hand, social connectedness puts individuals at greater risk or susceptibility for acquiring enteric pathogens via contact-mediated transmission. Yet conversely, in strongly bonded societies like humans and some nonhuman primates, having close connections and strong social ties of support can also socially buffer individuals against susceptibility or transmissibility of infectious agents. Using social network analyses, we assessed the potentially competing roles of contact-mediated transmission and social buffering on the risk of infection from an enteric bacterial pathogen (*Shigella flexneri*) among captive groups of rhesus macaques (*Macaca mulatta*). Our results indicate that, within two macaque groups, individuals possessing more direct and especially indirect connections in their grooming and huddling social networks were *less susceptible* to infection. These results are in sharp contrast to several previous studies that indicate that increased (direct) contact-mediated transmission facilitates infectious disease transmission, including our own findings in a third macaque group in which individuals central in their huddling network and/or initiated more fights were *more likely* to be infected. In summary, our findings reveal that an individual's social connections may increase *or* decrease its chances of acquiring infectious agents. They extend the applicability of the social buffering hypothesis, beyond just stress and immune-function-related health benefits, to the additional health outcome of infectious disease resistance. Finally, we speculate that the circumstances under which social buffering versus contact-mediated transmission may occur could depend on multiple factors, such as living condition, pathogen-specific transmission routes, and/or an overall social context such as a group's social stability.

Introduction

In humans and other animals, the strength and diversity of social relationships strongly influence the risk of acquiring infectious diseases (Alexander, 1974; Drewe & Perkins, 2015; McCowan et al., 2016; Nunn, 2012). In addition to life-history traits of the host, biology of the pathogen, and the degree of contact with contaminated environmental sources (Drewe & Perkins, 2015; Kappeler et al., 2015), social connections with group conspecifics may also influence the risk of acquiring and/or transmitting a pathogen (Drewe & Perkins, 2015). This may occur in two ways. On the one hand, increased connections may lead to greater chances of infection from pathogens via contact-mediated transmission (Drewe & Perkins, 2015), making infectious disease acquisition a major cost of social living (Alexander, 1974; Freeland, 1976; Loehle, 1995; MacIntosh et al., 2012). Yet social connections may also mitigate the impact of stressors or immunosuppressive effects of stress, thereby socially buffering an individual to decrease their susceptibility to infection (Hennessy et al., 2009; Kikusui et al., 2006; McCowan et al., 2016; Sapolsky, 2005; Sapolsky et al., 2000; Segerstrom & Miller, 2004; Young et al., 2014). To better understand the impact of social life on disease risk, it is necessary to characterize the potentially competing impacts of both greater contact-mediated transmission *and* social buffering on susceptibility to, and transmission of pathogens.

Traditional models of disease acquisition and transmission in social systems assume that individuals interact randomly (Anderson & May, 1992). In reality, individuals differ in how they navigate their social environment, which reflects differences in their social strategies to maximize fitness (Alexander, 1974; Bansal et al., 2007). Such heterogeneity can be modeled using social network analysis (Krause et al., 2007; McCowan et al., 2008). Most recent work implementing social network analysis to investigate infectious agent epidemiology in animal

systems has focused on contact-mediated infection and transmission (reviewed in (Drewe & Perkins, 2015)). Broadly, this phenomenon predicts that greater social connectedness, via more frequent contact rates with infected individuals within one's social network, increases one's risk of being infected by socially-transmitted pathogens (Drewe & Perkins, 2015). Specifically, being very central, and/or showing increased rates of contact-associated behaviors (e.g. social or allogrooming) within a social network has been shown to impact a variety of infectious-disease associated health outcomes, such as increasing endoparasite load, prevalence of a specific pathogen, and pathogen~~ic~~ diversity of individuals (Nunn, 2012). Indeed, contact-mediated transmission of pathogens has been shown to occur among a variety of animal social groups, such as group-living lizards (Godfrey et al., 2009), Tasmanian devils (Hamede et al., 2009), Belding's ground-squirrels (VanderWaal et al., 2013), and nonhuman primates (MacIntosh et al., 2012; Rimbach et al., 2015). Furthermore, epidemiologists speculate that highly central individuals may also act as *superspreaders* of pathogens because they can also transmit an infection to their neighbors (Drewe & Perkins, 2015; Lloyd-Smith et al., 2005). *Yet an individual's susceptibility versus resistance to infection may depend not just on its direct connections but also on its neighbors' connections* (Farine & Whitehead, 2015). To date, the impact of such indirect or secondary connections on infection risk has received relatively less attention in epidemiological studies (but see (MacIntosh et al., 2012)).

Even less explored is the possibility that having more direct or indirect connections in a network may, via *social buffering*, protect individuals from social and environmental stressors (Kaplan et al., 1991; Young et al., 2014), and *decrease* rather than *increase* their susceptibilities to infection. In societies where individuals maintain strong social bonds (e.g. humans, some nonhuman primates like baboons (*Papio sp.*) and macaques (*Macaca sp.*)), social buffering is

well-documented - having more social ties can mitigate stress to generate positive health outcomes (Cobb, 1976; Young et al., 2014) and indeed, enhance longevity and survival (Archie et al., 2014; Silk et al., 2010). In humans for example, having more connections who provide social support during times of conflict alleviates stress-levels (Janowski et al., 2012) and decreases susceptibility to non-communicable diseases such as cancer and cardiovascular disease (Uchino, 2004, 2009). In nonhuman primates, grooming (an affiliative social interaction) may be exchanged among group members for access to social support (Seyfarth, 1977), and is also known to lower circulating levels of glucocorticoids by reducing activation of the hypothalamic-pituitary-adrenal (HPA) axis (Young et al., 2014). Socio-positive interactions appear to be key in alleviating stress, which both enhances immune function (Sapolsky et al., 2000) and decreases susceptibility to infectious agents (Cohen et al., 2007; Segerstrom & Miller, 2004). Yet to our knowledge, no work has investigated the effects of social buffering on health using network-based analytical approaches, or in the context of infectious diseases. To better understand how social connections impact disease-related health outcomes, it is imperative to assess the effects of contact-mediated transmission and social buffering, particularly indirect social connections through which group members may elicit social support, on the risk of acquiring infectious agents.

Here we use a nonhuman primate model, the rhesus macaque (*Macaca mulatta*), to test whether the risk of infection from a bacterial pathogen (*Shigella flexneri*) is higher in socially well-connected individuals or lower in individuals buffered by having access to social support. Enteric bacterial pathogens, such as *Shigella*, are ubiquitous, well documented, and among the most communicable pathogens in humans (DuPont, 2000). Although infected individuals can occasionally remain asymptomatic, doses as low as between 20-200 organisms

120 may be sufficient to cause symptoms of Shigellosis, ranging from acute to severe diarrhea, fever,
121 and even death (DuPont, 2000). Shigella transmission in humans occurs via the fecal-oral route,
122 or person-to-person contact, as well as consumption of contaminated food and water sources
123 (Anderson & May, 1992; Scallan et al., 2011). Among nonhuman primates, *Shigella* (and indeed
124 other enteric bacteria) are surprisingly understudied in comparison with other pathogens
125 (www.mammalparasites.org) (Nunn & Altizer, 2006). The few studies to date report low
126 prevalence in some free-living populations that live in close proximity to humans (e.g. rhesus
127 macaques: (Beisner et al., 2016); Savannah baboons: (Drewe et al., 2012; Harper et al., 2012)).
128 Although the dosage levels of *Shigella flexneri* that may generate virulent effects among
129 primates is unclear, the occurrence of acute enteritis in rhesus macaques housed in captivity has
130 been linked to infection this pathogen (Lee et al., 2011). ~~More generally~~ there have also been
131 two documented outbreaks of Shigellosis among ~~a~~ semi-free ranging rhesus macaques in Puerto
132 Rico, resulting in enteritis, abortions, and increased mortality rates among pregnant monkeys
133 (Kessler & Rawlins, 2016). Such documentations of virulent infection among primates, further to
134 its well-established social- and/or environmental-contact mediated transmission routes among
135 humans, make *Shigella* an *ideal* pathogen for investigating the effects of social network
136 connectedness on susceptibility versus resistance to infection risk.

137 Rhesus macaques are biologically, socially and cognitively analogous to human societies
138 (Cobb, 1976; Suomi, 2011). They live in large (~20-150 individuals), multi-male-multi-female
139 social groups (Thierry, 2007). Individuals maintain and reinforce their social relationships using
140 a variety of behaviors, such as aggression, grooming, and social huddling (Lindburg, 1971; Sade,
141 1972). These behaviors are heterogeneously distributed in accordance with sex, kinship, age-sex
142 category, and dominance ranks of individuals (Thierry, 2007). For example, social bonds

established via grooming tend to be strongest among closely related females, and/or females with adjacent dominance rank which is also inherited in accordance with kinship (e.g. (Berman & Thierry, 2010; Chapais, 2006; Sade, 1972)). From a health perspective, these behaviors typically bring individuals into physical contact and can also influence levels of social tension or stress. For example, grooming has well-documented fitness, particularly stress-relieving, benefits in nonhuman primates (summarized in (Henzi & Barrett, 1999)) as well as in other animal taxa (e.g. social insects: (Moore et al., 1995) horses: (Kimura, 1998)). Similarly, although the functional significance of huddling among macaques is yet to be documented, this behavior may be considered to be physiologically (involving relaxed body-to-body contact) analogous to hugging among humans, which has been shown to socially buffer individuals from infection by the common-cold virus (Cohen et al., 2015). Conversely, grooming can also serve as a contact-route of infection (Japanese macaques (*M. fuscata*: (MacIntosh et al., 2012); other primates such as brown spider monkeys (*Ateles hybridus*: (Rimbach et al., 2015)). Aggressive interactions have the potential to enhance susceptibility to infection by bringing aggressors and recipients into physical contact (Drewe, 2010a), or by elevating stress-levels in recipients of aggression (e.g. (Muller & Wrangham, 2004)). Among Japanese macaques (MacIntosh et al., 2012) revealed that dominance rank, an outcome of dyadic aggressive interactions, positively affected parasite diversity but not fecal glucocorticoids, indicating a contact- rather than a stress-mediated infection-route. In primate species like rhesus macaques that are characterized by despotic social relationships, the effect of dominance rank on infection risk may be somewhat redundant to examining those of social network connectedness, since social rank directly effects such connectedness and centrality (e.g. (MacIntosh et al., 2012; Sueur et al., 2011b)). Yet other than (MacIntosh et al., 2012), we are unaware of any work that has simultaneously examined the

166 effect(s) of greater network connectedness (which may occur via dominance rank) to specifically
167 evaluate the contrasting roles of contact-mediated transmission *and* social buffering, on
168 infectious ~~agent~~ risk.

169 We first establish *(1) the prevalence of Shigella flexneri within each of three groups of*
170 *captive rhesus macaques.* For each of these groups, we next reconstruct each of three types of
171 social networks based on grooming, huddling, and aggressive interactions respectively. Upon
172 doing so, we investigated whether *(2) greater centrality and/or social connectedness through*
173 *both direct and (where relevant) indirect or secondary connections in these networks mitigated*
174 *macaques' risk of infection via social buffering, versus enhanced this risk through contact-*
175 *mediated transmission. Specifically, if connections socially buffer individuals against Shigella*
176 *infection risk, we predicted that this bacterial pathogen will be the least prevalent among*
177 *individuals with the most direct and/or indirect social connections. On the other hand, if*
178 *possessing connections enhance infection risk via contact-mediated transmission, we predicted*
179 *that infection will be most prevalent among individuals with the highest number and/or diversity*
180 *of direct connections.* Finally, we also ascertained whether *(3) attributes of individuals other*
181 *than their network positions, but which may influence their positions (particularly sex,*
182 *dominance rank, and the certainty of their dominance relationships ((Fujii et al., 2014); see*
183 *Methods)), also influenced their risk of infection. Specifically, we asked whether Shigella*
184 *infection was least likely among high-ranking females who typically form the strongest social*
185 *bonds. Further, given that increased uncertainty in dominance relationships could be more*
186 *stressful (Vandeleest et al., 2016), we asked whether the effect of possessing increased*
187 *connections on socially buffering individuals from infection risk was more clearly discernible*
188 *among individuals who also had more uncertain compared to certain dominance relationships.*

Materials and Methods

Study location and subjects: The study was conducted at the California National Primate Research Center (CNPRC) and the School of Veterinary Medicine (SVM), University of California at Davis. The subjects were 299 adult rhesus macaques (90 males, 209 females) between the ages of 3 and 29 (mean = 7.7 years), distributed across three social groups (101 in Group I, 96 in Group II, and 102 in Group III). The groups were housed in 0.2 ha outdoor enclosures containing multiple A-frame structures, suspended barrels, swings, and perches; they were free to engage in social interaction. Animals were fed a standard diet of monkey chow twice per day at approximately 0700 h and between 1430 and 1530 h. Fresh fruit or vegetables were provided one time per week and seed mixture provided daily. Water was available *ad libitum*. The outdoor housing facilities are exposed to a minimum level of disturbance, and may be considered semi-naturalistic. The protocols used for this research were approved by the UC Davis Institutional Animal Care and Use Committee (IACUC), and were in accordance with the legal requirements of the jurisdictions in which the research was conducted.

Behavioral Data Collection: Behavioral data were collected on each group for 6 weeks, two groups in the spring (Group I: March-April 2013; Group III: March-April 2014) and one in the fall (Group II: September-October 2014). For each group, three observers collected data for 6 hours on 4 days per week from 0900-1200 h and 1300-1600 h, using (i) an Event Sampling design for aggressive interactions, and (ii) Scan Sampling for affiliative grooming and huddling interactions (Altmann, 1974). These have been shown to optimize reliable collection of data in large groups of subjects (McCowan et al., 2011), both improving statistical power and circumventing nonindependence issues that may affect the computation of reliable social

network metrics (Farine & Whitehead, 2015). For each 'event', the identities of the initiator and recipient macaque, and their specific behavior(s) were recorded. Grooming was defined as an animal cleaning or manipulating the fur of another individual. Huddling was defined as the occurrence of all forms of body-contact, including (but not restricted to) ventral contact or an embrace between two individuals that did *not* involve a social behavioral interaction (e.g. grooming, contact aggression). Aggression was categorized according to severity and included threat (open mouth stare, brow flash, ear flap), mild aggression (threat and follow, lunge, push, slap, chase < 6 meters), moderate aggression (grapple, wrestle, chase > 6 meters), and intense aggression (pin or bite). We recorded events of polyadic aggression as a series of dyadic interactions. Data on all dyadic and polyadic interactions were used to calculate the network metrics of degree and strength. Submission categories included freeze/turn away, move away, run away < 6 meters, run away > 6 meters, and crouch. For computing dominance rank and certainty (see below), we used only data on dyadic aggressive and submissive interactions.

Social Network and Dominance Metrics: For each of the three groups, we constructed three types of social networks: (1) *groom*, (2) *huddle*, and (3) *aggression*. From undirected grooming and huddling networks, we calculated the number of direct connections (or *degree centrality*), the total number of shortest pathways between other pairs of animals that pass through an individual (or *betweenness centrality*), and a metric of social capital that takes both direct and indirect connections of individuals into consideration (*eigenvector centrality***)** (Farine & Whitehead, 2015; Makagon et al., 2012). For directed networks of grooming and aggression, we also calculated the number of connections given and received (*in-* and *out-degree* respectively), and the strengths of these connections weighted by the frequencies of interactions (*in-* and *out-strength* respectively). These were computed using the *Statnet* and *Sna* (Handcock et

al., 2006) packages in R. Given its estimation of an individual's direct connections, *degree centrality* has been extensively used in epidemiological studies to date that have focused on contact-mediated pathogen transmission (Drewe, 2010a; Drewe & Perkins, 2015; Rimbach et al., 2015). On the other hand, in- and out-degree and strength in grooming may also be indicative of reduced infection risk via social buffering, given that grooming interactions have well-documented benefits of lowering the physiological stress levels among both givers and receivers (Henzi & Barrett, 1999; Smutt et al., 2007; Young et al., 2014). Although *betweenness centrality* has been less commonly used in epidemiological studies, it has been proposed as being key metric in predicting *the potential* for the flow of infectious agents through the generally more densely connected social networks (Drewe & Perkins, 2015; Farine & Whitehead, 2015; Newman, 2005; VanderWaal et al., 2014). Finally, as an indicator of access to social support via indexing secondary connections, *eigenvector centrality* may be particularly key in determining whether the possession of such extended support circles beneficially inhibits (via buffering), versus detrimentally exposes an individual to infection risk (via contact-transmission: e.g. (MacIntosh et al., 2012)).

We implemented a recently developed network algorithm, Percolation-and-Conductance (or *Perc*: (Fujii et al., 2014)), to compute dominance ranks and certainties from aggression networks constructed from dyadic interactions only. This method combines information from direct dominance interactions with information from multiple indirect dominance pathways (via common third parties) to quantify dyadic dominance relationships (Fujii et al., 2014). This algorithm identifies all potential flow pathways in the network, weighting the contribution of each path to the imputed matrix by its likelihood of being traversed by the random walk. This method yields two outputs: a matrix of dyadic dominance certainty values (range: 0-1) and the

lowest-cost linear rank order. The former represent the cumulative information from all network pathways between each pair of animals. A dominance certainty value of 1 reflects the highest possible certainty that the row animal outranks the column animal (and 0 reflects the highest possible certainty that the column animal outranks the row animal) whereas 0.5 means the dominance relationship is perfectly ambiguous. To compute the average dominance certainty of each individual, we transformed all dyadic dominance certainty values between 0.5 and 1, and calculated the row-wise average for each animal. We also transformed ordinal dominance ranks for each group into the proportion of animals outranked within their respective groups (i.e. 0 is the lowest ranked animal and 1 is the highest ranked animal). This measure of 'percentile ranks' was used in the place of ordinal ranks in the analyses.

Pathogen Characterization: Prior to fecal collection, animals were immobilized (10 mg/kg of ketamine) and given standard physical examinations by veterinary staff (e.g., checked for injuries, weighed). We collected two fresh fecal swabs from every macaque at the end of the behavioral observation period following previously published methods (Good et al., 1969). Briefly, a sterile cotton-tip swab was inserted into the rectum of each individual, rotated gently to collect fecal material, and immediately immersed into a 15 ml test-tube (labeled with the animal ID) containing sterile Tryptic Soy Broth (TSB; BD Franklin Lakes, NJ, USA); a duplicate sample was taken using another sterile swab and placed into a second TSB tube. The samples were incubated within 4 hours of collection; the tubes were incubated with orbital rotation of 100 rpm at (1) 25°C for 2 h, (2) 42°C for 8 h, and (3) held static at 6°C overnight. *Shigella* was isolated from the TSB enrichment; ~10 µL enrichment was struck for isolation onto MacConkey agar plates (BD Franklin Lakes, NJ USA), and incubated at 37°C for 18-24 hours. Suspect colonies were further isolated and characterized on Xylose Lysine Deoxycholate agar plates

(XLD, BD Franklin Lakes, NJ USA). Two isolated colonies per sample were biochemically confirmed using (i) Triple Sugar Iron (TSI) (Remel Lenexa, KS), (ii) Citrate (Remel Lenexa, KS USA), (iii) Urea (BD Franklin Lakes, NJ USA), and (iv) the Methyl Red – Voges-Proskauer (MR-VP) test (BD Franklin Lakes). Individuals which tested positive from at least one of the two swabs were categorized as *infected*, with those that tested negative from both swabs deemed *uninfected*.

Data analysis: The low prevalence of bacterial infection among macaques (e.g. (Beisner et al., 2016; Drewe et al., 2012); see Results) has the potential to impact the statistical power of our analyses. We therefore conducted some diagnostic assessments to determine whether there was sufficient homogeneity of the data across groups to warrant combining them in a single model-set. Diagnostic plots (Figure 1) showed that while the patterns of relationships between *Shigella* infection and network metrics appeared highly similar for Groups I and II, those for Group III were starkly different. These are further supported by scatter-plots that showed similar infection patterns (concentrated among individuals moderately or least central in their grooming and huddling networks) in Groups I and II, but a different pattern of infection being widely distributed across individuals of varying centralities in Group III (Supporting Figure 1). Finally, Groups I and II were highly similar, and different from Group III, in the age structure of individuals, time of formation, and the number of matrilines, any or all of which may impact heterogeneity in social network dynamics (Supporting Table 1; see Discussion). For these reasons, we chose to combine Groups I and II into a single, homogeneous population for our analyses, and analyzed Group III as a second, separate dataset.

To investigate our aims, we implemented an Information-Theoretical approach to construct generalized linear mixed-effects models (Burnham et al., 2011; Grueber et al., 2011;

Whittingham et al., 2006). For each of the two datasets, we constructed 48 models using the lme4 package in R (Bates et al., 2016). We used a binomial distribution with a Logit link function to examine the effect of social network metrics, attributes, and their potential interactions on *Shigella* infection across individual macaques (McCullagh & Nelder, 1989), Given the potential collinearity or non-independence among centrality network metrics (Farine & Whitehead, 2015; Krause et al., 2007; MacIntosh et al., 2012; Sueur et al., 2011a), we refrained from using the automated 'Dredge' function that provides a full set of sub-models for a set of predictors (Grueber et al., 2011). Instead, our *complete model-set* (48 models for each dataset: Supporting Table 2) was composed of individually-constructed models, specifically a 'null' model (1 model), a model each for the main effect of each of nine network metrics (11 models), combinations of two uncorrelated network metrics (grooming or huddling with aggression) to examine their combined main effects (28 models), main effects plus a sex × dominance rank interaction (1 model), and main effects plus an interaction between each groom or huddle centrality metric and dominance certainty (7 models). From these, we selected our *candidate model-set* by selecting all models with a dAICc (or ΔAIC) < 2 (Burnham et al., 2011), and using the law of parsimony to eliminate models whose increased complexity does not improve AICc over a simpler model in the candidate set (Burnham et al., 2011; Grueber et al., 2011; Richards, 2005, 2008). We report coefficients and other summary statistics ($P = 0.05$ as 'significant', $0.5 < P < 0.1$ as a 'nonsignificant trend') from each model within our candidate model-sets.

Results

Prevalence of Shigella flexneri in rhesus macaques: From the biochemical assays, we detected a moderate-low prevalence of *Shigella* among the CNPRC macaques. Specifically, Group I had the highest prevalence, with 23 out of 101 individuals being infected (~23%). Groups II and III showed similarly low prevalence, i.e. 8 out of 96 (~8%) and 7 out of 100 (7%) sampled individuals respectively.

Infection, Network Metrics, and Individual Attributes: *In groups I and II, we found that well-connected individuals were socially buffered against the risk of Shigella infection***.** There were four models in the candidate model-set for the analysis of groups I and II (Table 1). These revealed significant, negative relationships between *Shigella* infection and three network metrics -- groom out-degree (Model 4), groom eigenvector centrality (Model 7; Figure 2), and huddle betweenness (Model 9) (Table 1). In other words, infection was least likely among individuals with strongest grooming and huddling connections. Further, there was a significant interaction between huddling betweenness and dominance certainty (Model 20). When explored further, we found that individuals with categorically *low* certainties (below the 50th percentile) showed strong, negative relationships between huddling betweenness and infection ($\beta = -4.19$, $p = 0.02$) whereas those with categorically *high* dominance certainties showed no such effects ($\beta = 0.38$, $p = 0.82$). In other words, social buffering via huddling connections was more discernible among individuals with low dominance certainties than those with high dominance certainties. Finally, models that included aggression network metrics and/or sex interacting with dominance rank were not part of the candidate model set (Table 1; Supporting Table 2), suggesting no clear relationships between these variables and infection.

In Group III, we found that Shigella infection was most prevalent among individuals that were well-connected, suggesting contact-mediated transmission. The candidate model-set

349 constituted four models (Table 2) that revealed significant, positive relationships between
350 *Shigella* infection and both aggression out-degree (Models 42, 11; Figure 3, 4) and out-strength
351 (Models 41, 13). There was also a non-significant positive trend between infection risk and
352 huddle betweenness (Models 41, 42) (Table 2; Figure 4). When we examined metrics from
353 aggression networks with only intense aggressive interactions (bites, attacks, i.e. those involving
354 contact), the positive relationships were sustained but nonsignificant (Model 42: contact-
355 aggression out-degree: $\beta = 3.37$, $p = 0.07$; huddle betweenness: $\beta = 2.87$, $p = 0.08$). Finally,
356 models that included grooming network metrics and/or sex interacting with dominance rank were
357 not part of the candidate model set (Table 2; Supporting Table 2), suggesting no clear
358 relationships with infection.
359
360 **Discussion**
361 In animal societies, the way in which sociality impacts individual health and fitness
362 remains hotly debated. In regards to infectious disease susceptibility, increased social
363 connections among group members may either facilitate the acquisition and transmission of
364 pathogens via social contact (Drewe, 2010b; Drewe & Perkins, 2015; Freeland, 1976; Loehle,
365 1995; MacIntosh et al., 2012), or may inhibit such acquisition via socially buffering individuals
366 against daily stressors to reduce the risk of environmental acquisition of pathogens (Cohen et al.,
367 2015; Hennessy et al., 2009; Kaplan et al., 1991; Young et al., 2014). Results from our study
368 speak to *both* of these processes. Specifically, they reveal that among two groups of rhesus
369 macaques (a species characterized by strong social bonds (Sade, 1972)), having greater social
370 connections in both grooming and huddling social networks socially buffered individuals against
371 the risk of infection from an enteric pathogen, *Shigella flexneri.* Yet in a third group, we found

372 that individuals who gave more aggression and possessed more huddling connections were more
373 likely to be infected, supporting the contact-mediated transmission hypothesis.
374 Nonhuman primates harbor a variety of pathogens, several of which also infect humans
375 (Engel & Jones-Engel, 2011; Jones-Engel et al., 2005; Kaur & Singh, 2009; Nunn, 2012; Nunn
376 & Altizer, 2006). In comparison with viruses and ectoparasites, the prevalence of zoonotic
377 bacteria are relatively understudied (Nunn & Altizer, 2006). We found a moderate-to-low
378 prevalence of *Shigella flexneri* in captive rhesus macaques, which is consistent with previous
379 studies of bacterial prevalence in other free-living primate populations (e.g. free-living rhesus
380 macaques: (Beisner et al., 2016), savannah baboons (*Papio anubis*: (Drewe et al., 2012)). Such
381 comparisons reflect the general dearth of studies on bacterial prevalence among captive animals;
382 on which studies to date have mostly focused on the clinical bases, i.e. the virulent versus
383 asymptomatic effects, of infection among individuals (Lee et al., 2011; Shipley et al., 2010).
384 Indeed, the last documented reports of group- or population-specific prevalence levels of enteric
385 bacteria among captive primates were on rhesus (23% of 4476 individuals: (Good et al., 1969))
386 and longtailed macaques (18.8% of 1297 individuals: (Takasaka et al., 1964)) imported into
387 biomedical facilities during the 60s. Given the now well-established, reliable characterization
388 approaches (see Methods), our reports of low prevalence were not likely artifacts of
389 Methodological issues. Among humans on the other hand, reports of enteric bacterial prevalence
390 are often *inflated* by sampling paradigms that focus purely on hospitalized patients, and/or
391 individuals already showing signs of symptoms (e.g. (Kotloff et al., 1999)). We contend that pre-
392 symptomatic, epidemiologically accurate assessments of enteric bacterial prevalence levels are
393 imperative both for humans and captively housed animals given that these pathogens are (i)
394 omnipresent, (ii) both socially and environmentally transmittable, and/or (iii) may cause

unpredictable outbreaks of virulent infection both among humans and macaques (DuPont, 2000; Gupta et al., 2004; Kessler & Rawlins, 2016). Among the CNPRC macaques, a logical next step would be to establish associations between ~~dosage levels of~~ *Shigella* infection and symptomatic effects (e.g. diarrhea, enteritis) that may necessitate clinical treatment.

Animal studies that demonstrate the impact of social buffering on health and stress mitigation have traditionally focused on stress-related health outcomes in pair-housed or monogamous species (reviewed in (Hennessy et al., 2009)), with evidence among socially cohesive, group-living species with strong social bonds emerging only more recently (e.g. Chacma baboons (*Papio hamadryas ursinus*): (Archie et al., 2014; Silk et al., 2010); Barbary macaques (*M. sylvanus*: (Young et al., 2014); humans: (Holt-Lunstad et al., 2010)). Our findings extend the impact of social buffering to infectious agent acquisition. Among two groups of rhesus macaques, our candidate models revealed that individuals possessing direct and secondary grooming connections, and strong huddling connections particularly among individuals with uncertain dominance relationships, were the least prone to infection from *Shigella flexneri*. Broadly, these findings add novelty to epidemiological studies implementing social networks by contradicting the popularly prevailing notion that infectious agent acquisition is a consistent *drawback* of group living (Alexander, 1974; Freeland, 1976; Loehle, 1995; MacIntosh et al., 2012). The negative relationship between *Shigella* infection risk and each of grooming outdegree and eigenvector centrality points to grooming being a strong source of social buffering. This is consistent with prior research among primates that has revealed that both giving and receiving grooming may mitigate social stress (Henzi & Barrett, 1999; Smutt et al., 2007). Further, the establishment of the social buffering phenomenon in a second type of affiliation network

(huddling) improves on most epidemiological studies that focus on just one (reviewed in (Drewe & Perkins, 2015)).

Specifically, the effect of grooming outdegree suggests that giving grooming may be more beneficial in lowering infection risk than receiving grooming, via potentially functioning to elicit additional buffering-related benefits such as social support (Seyfarth, 1977; Smutt et al., 2007). This argument is further supported by the effect of grooming eigenvector centrality, i.e. having a well-connected social circle of primary *and* secondary connections to elicit such support (Farine & Whitehead, 2015), on also lowering infection risk. Surprisingly, eigenvector centrality has been frequently overlooked in infectious disease research in favor of metrics based on direct connections (Drewe & Perkins, 2015). Yet one study (MacIntosh et al., 2012) found that grooming eigenvector centrality *increased* likelihood of infection from a nematode parasite (*Strongyloides fuelleborni*) in wild Japanese macaques, suggesting contact-mediated transmission. Our finding revealed an opposite, social buffering-mediated effect. Such an effect may be particularly discernible in study systems, such as large groups of captive macaques, that are socially (in addition to biologically: (Suomi, 2011)) analogous to dense human communities in urban settings wherein having a broad circle of social connections also has well-documented health- and fitness-related benefits (Janowski et al., 2012; Uchino, 2004, 2009).

In addition to grooming connections, individuals with higher huddling betweenness were also more socially buffered against infection. Where social buffering prevails, betweenness, which measures the relative number of pathways that pass through each individual, may be a more reliable indicator of the strength and extent of support ties in networks (such as huddling) where the direction of the relationship (unlike in grooming) is less significant. Further, the effect of huddling betweenness in mitigating infection risk was particularly strong among individuals

with more uncertain dominance relationships. Such a complex relationship between dominance status, network connectedness, and infection risk is consistent with what we see in the literature. The effect of dominance status on infection risk varies by social system and the type of health outcome examined. In primate groups for instance, high social rank is related to greater access to grooming partners and may enhance contact-associated susceptibility to infectious diseases (MacIntosh et al., 2012). Further, the relationship between dominance rank and stress (a proxy for infectious disease susceptibility via social buffering (Capitanio & Cole, 2015)) is highly inconsistent, with both high- and low-ranking individuals experiencing different types of social and biological stressors (e.g. (Abbott et al., 2003; Sapolsky, 2005), but see (Foerster et al., 2015)). Thus dominance status, rather than linearly predicting infection risk, may instead interact with stress-levels and/or social network connectedness to influence this risk. To assess dominance status, we use the measure of certainty in addition to rank, which had no effects. Dominance certainty differs from rank; it is a metric of the *predictability* of the direction of an individual's dominance interactions and pathways of interactions, irrespective of the outcome of wins or losses (Fujii et al., 2014). Biologically, it may be a more important moderator (than rank) on health outcomes. For instance, a recent study on the CNPRC rhesus macaques showed that individuals that face greater unpredictability in their dominance encounters also showed pronounced biomarkers of poor health, including inflammatory proteins and diarrhea (Vandeleest et al., 2016). Under such circumstances, the beneficial impact of possessing strong social connections in buffering individuals against infection risk may be more clearly discernible. A clearer picture on the effects of dominance on infection risk may emerge when future studies examine the effects of dominance certainty instead of or in addition to rank.

462 Our findings should lead to future investigations of whether increased network
463 connectedness mitigates biological stress indicators (e.g. Glucocorticoid or GC content:
464 (Sapolsky et al., 2000; Young et al., 2014)) and indeed, other systemic inflammation markers
465 (e.g. C-reactive protein (CRP), Interleukin-6 (IL-6): (Libby et al., 2002)) that may influence
466 buffering-mediated infection risk, which are currently under analysis. It is also conceivable that
467 social connections may impact susceptibility versus resistance to bacterial infection via altering
468 individuals' foraging regimes and thereby modifying their gut microbial flora (Degnan et al.,
469 2012; McCord et al., 2014). Among captive long-tailed macaques (*M. fascicularis*), for instance,
470 commensal gut *E. coli* competitively inhibited infection from *Shigella* (Seekatz et al., 2013).
471 Thus, assessing the links between social networks and stress- and/or microbiome-mediated
472 infection risk would be logical next steps.
473 Social networks have proved to be highly beneficial in detecting contact-mediated
474 infection and transmission of pathogens (Drewe & Perkins, 2015). Consistent with these
475 previous efforts, we found that giving more aggression to others and having more huddling
476 connections both increased individuals' risk of *Shigella* infection in our third rhesus macaque
477 group. Aggression may increase the risk of infection either via contact-risk (Drewe, 2010a,
478 2010b), or via weakening the health-related benefits of social buffering (Muller & Wrangham,
479 2004; Ostner et al., 2008). The fact that aggression given, but not received, was related to
480 infection risk better supports the former compared to the latter. Further, the positive association
481 between huddling betweenness and infection risk suggests that individuals with increased
482 huddling connections may be *superspreaders* of pathogens (Lloyd-Smith et al., 2005). This is
483 because when contact-transmission is prevalent, betweenness, in its calculation of the relative
484 number of pathways that pass through a node, specifically measures the potential for the flow of

485 information (or pathogens) through a node (Drewe & Perkins, 2015; Farine & Whitehead, 2015;
486 Newman, 2005; VanderWaal et al., 2014). It may therefore be an especially useful metric to
487 parse out 'informative' nodes among captive groups where individuals tend to come into contact
488 with more individuals than those in free-living groups, which are less spatially constrained
489 (Drewe & Perkins, 2015; Griffin & Nunn, 2012). Finally, our findings yielded no information on
490 *Shigella* transmission through Group III's grooming network. This might be because grooming
491 involves a more subtle form of hand-to-body-hair contact compared to huddling, which involves
492 more direct and prolonged body-to-body contact that may increase the chances of transmission.
493 Although contact-mediated transmission in the aggression network might be expected to
494 be most pronounced in a network composed of only intense contact-aggression, we found the
495 reverse: a weaker association. One explanation for this could be that the detectability of
496 transmission may be influenced by pathogen-specific differences in modes of transmission. For
497 instance, the transmission of viral pathogens from macaques to humans would require the
498 occurrence of specifically intense forms of contact, such as bites and scratches, which may
499 culminate in the contact-exchange of body-fluids such as blood and saliva (Engel & Jones-Engel,
500 2011; Engel et al., 2013). Yet simpler body-contact and/or the sharing or consumption of
501 contaminated food or water source has been shown to be sufficient for the transmission of enteric
502 bacterial pathogens (like *Shigella*) among humans (Benjamin et al., 2013; Cooley et al., 2007).
503 In social systems, multiple additional factors may determine whether social buffering
504 versus contact-mediated transmission of infectious agents may prevail. To our knowledge, most
505 epidemiological studies that have reported contact-mediated transmission of infectious agents
506 through social networks have focused on free-living or wild animal groups (reviewed in (Drewe
507 & Perkins, 2015)). It is conceivable that social buffering may be more readily discernible among

large, spatially constrained primate groups living in captivity. As in dense, suburban populations of humans (e.g. (Janowski et al., 2012; Uchino, 2004, 2009); see above), such living conditions manifest in more by-standing to witness (De Marco et al., 2010), and/or less opportunities for the social avoidance of, agonistic encounters (Fujii et al., 2014; McCowan et al., 2008). This may amplify the utility of strong social ties as avenues of support or stress-relief.

Yet by itself, living condition does not explain the heterogeneous pattern observed in Group III, which showed evidence for contact-mediated transmission. We offer two potential explanations for this. First, unlike Groups I and II which had diverse age-structures (3 – 22 year olds), Group III was primarily composed of younger individuals (3 – 11 years of age) (Supporting Table I). Among free-ranging rhesus macaques, age proximity has been shown to positively influence the quality of affiliative social relationships (Widdig et al., 2001). Similarly, it may be likely that social ties among younger animals, on account of being more nascent and/or unpredictable, are not of the relationship quality that may be required for social buffering. Second, the discernibility of social buffering versus contact-transmission may be governed by higher-order social contexts, such as group stability. For instance, both Groups I and II maintained consistent dominance relationships with few reversals, as evidenced by consistencies in the overall directions of dominance encounters across their aggression and submissive status networks (Chan et al., 2013). In Group III, a comparison of these networks revealed marked inconsistencies in the direction of the relationships, which persisted until the group suffered a social collapse around 13 weeks after the data collection period (Chan et al., 2013). Thus it is conceivable that in captivity, contact-mediated transmission is more easily decipherable under socially unstable conditions where the effect of social buffering is minimal or absent. Validations of both these explanations await future assessments of (i) the age-classes of immediate

connections, or "neighborhoods", of older versus younger individuals, and (ii) expansions of current findings to additional groups.

In summary, this study suggests that within captive housed groups of a nonhuman primate biologically and socially analogous to humans, individuals' network connections may socially buffer them against, *or* promote the contact-mediated transmission of infection from an enteric bacterial pathogen. The generality of these findings awaits expansions of similar approaches to additional social and pathogenic taxa. Such efforts may facilitate delineating what may be a fine line between when/ how social network connections may be beneficial versus detrimental to infectious disease risk.

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826

Figure 1(on next page)

Diagnostic plots of networks metrics and *Shigella* infection

Diagnostic plots of between-group similarities and differences in relationships between network metrics and Shigella infection. In each plot, the mean value of a network metric is plotted for infected and uninfected individuals within each group

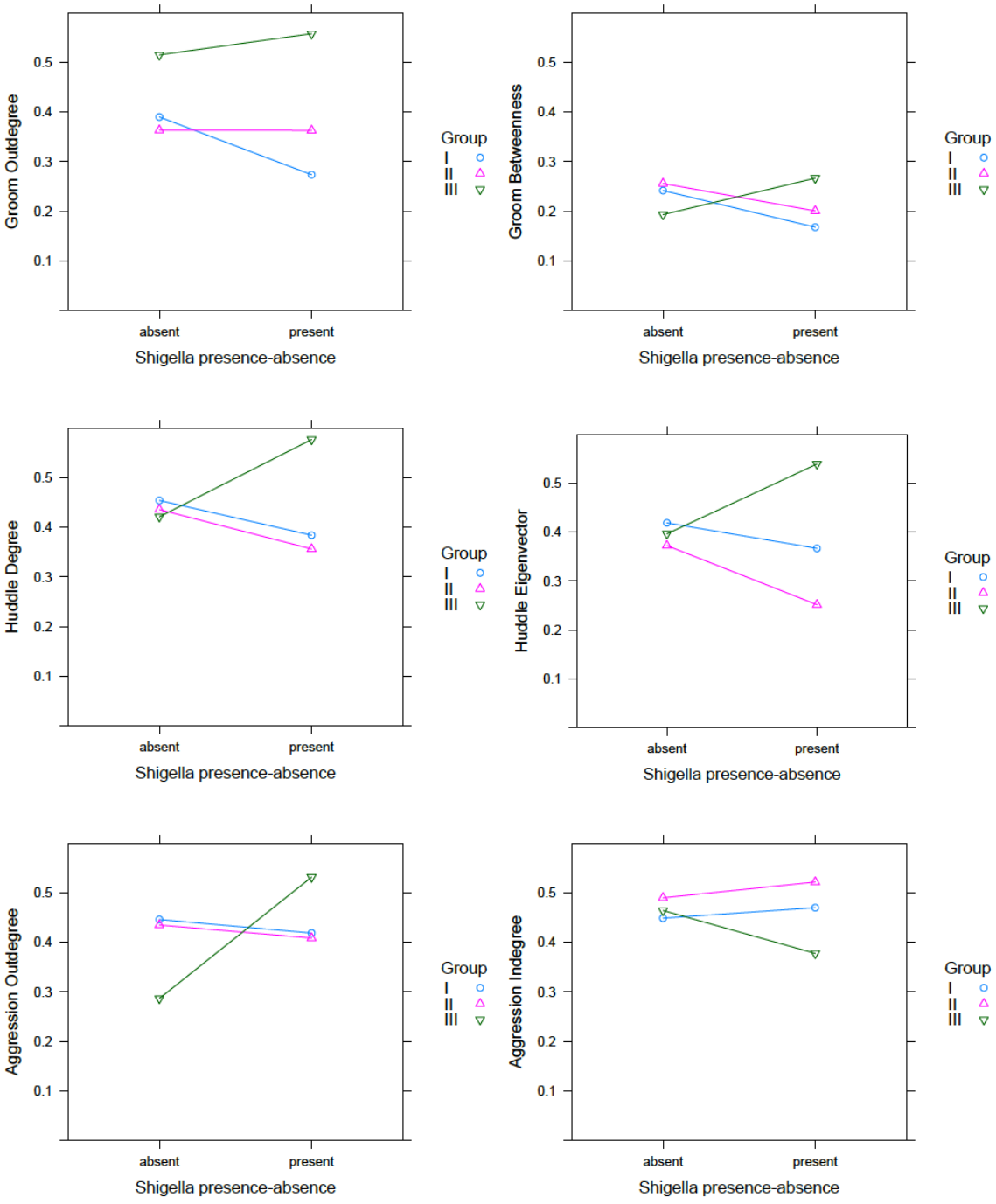
Groom Outdegree
Groom Betweenness
Huddle Degree
Huddle Eigenvector
Aggression Outdegree
Aggression Indegree
0.1
0.2
0.3
0.4
0.5
absent
present
Shigella presence-absence
Group
I
II
III

Figure 2(on next page)

Group I: Grooming Social Network and *Shigella* Infection Risk

Social Network graph of grooming relationships indicating the effect of social buffering on Shigella infection in Group I. Nodes are sized proportional to the eigenvector centralites of individual macaques. Infection (yellow nodes: N = 23 out of 101) is restricted to individuals with ~~the lowest~~ eigenvector centralities

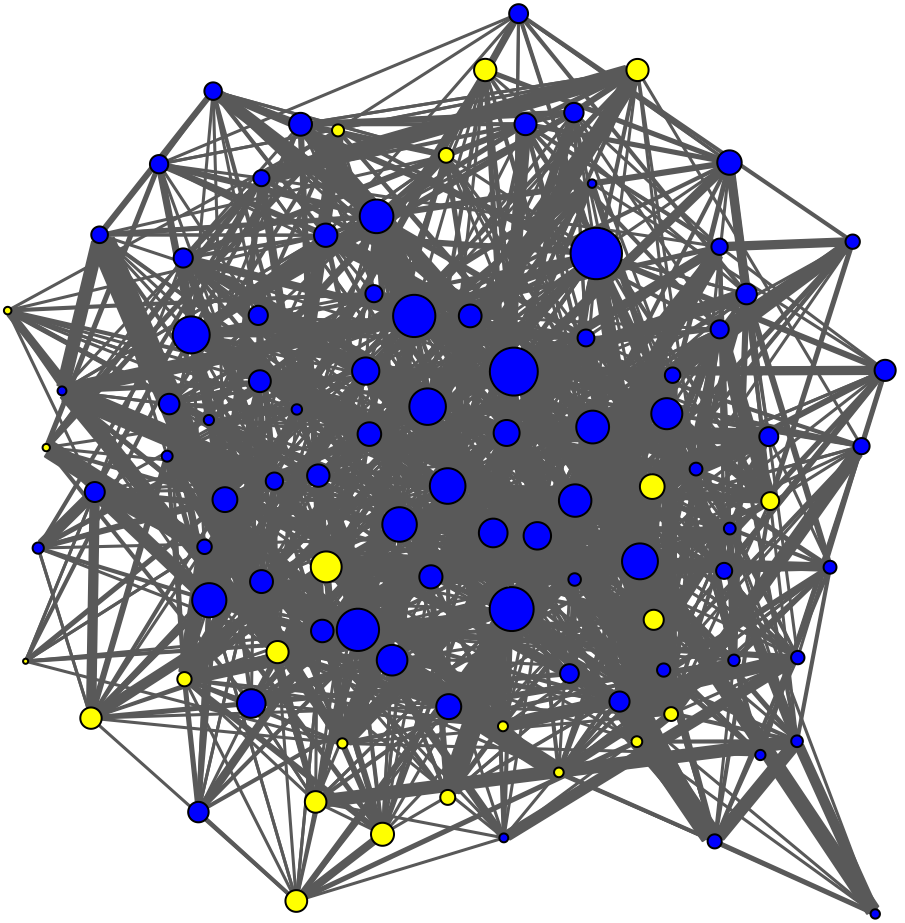

Figure 3(on next page)

Group III: Aggression social network and *Shigella* infection risk

Social Network graph of aggression relationships indicating the effect of social contact on Shigella infection in Group III. Nodes are sized proportional to the aggression out-degrees of individual macaques. Infection (yellow nodes: N = 7 out of 100) is prevalent among individuals with moderate-to-high out-degree

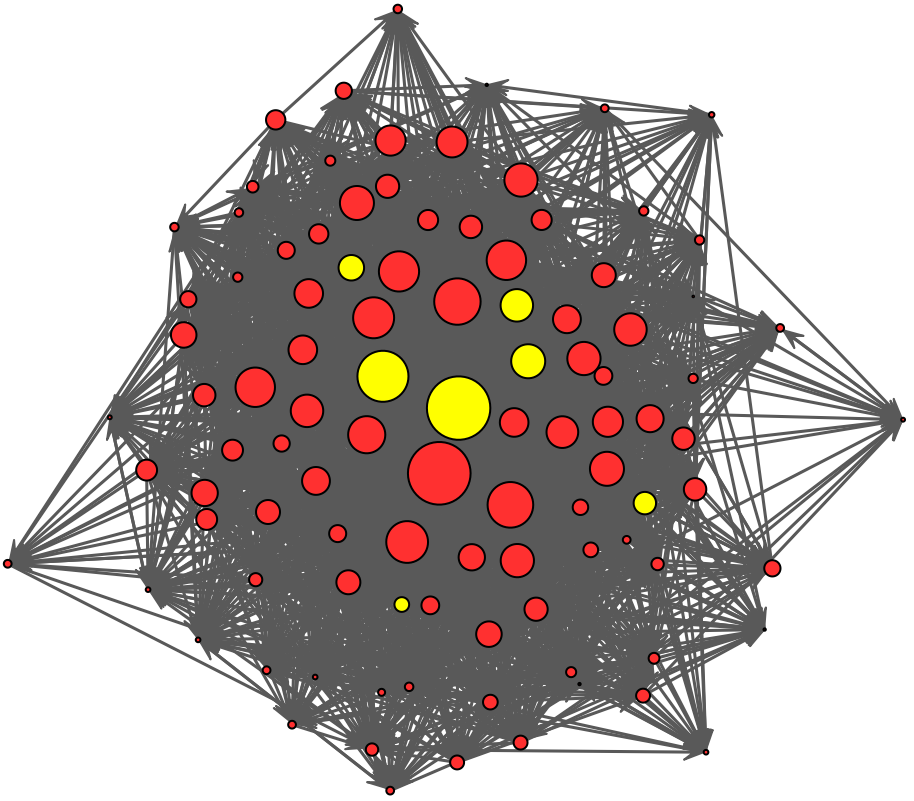

Figure 4(on next page)

Group III: Aggression, huddling, and *Shigella* infection risk

Scatter-plot showing the effect of social contact on *Shigella flexneri* infection for Group III. Black dots are infected macaques (N = 7 out of 100), and are concentrated among those with moderate-to-high huddle betweenness *and* aggression out-degree

Groups III: Huddling, Aggression, & Shigella Infection

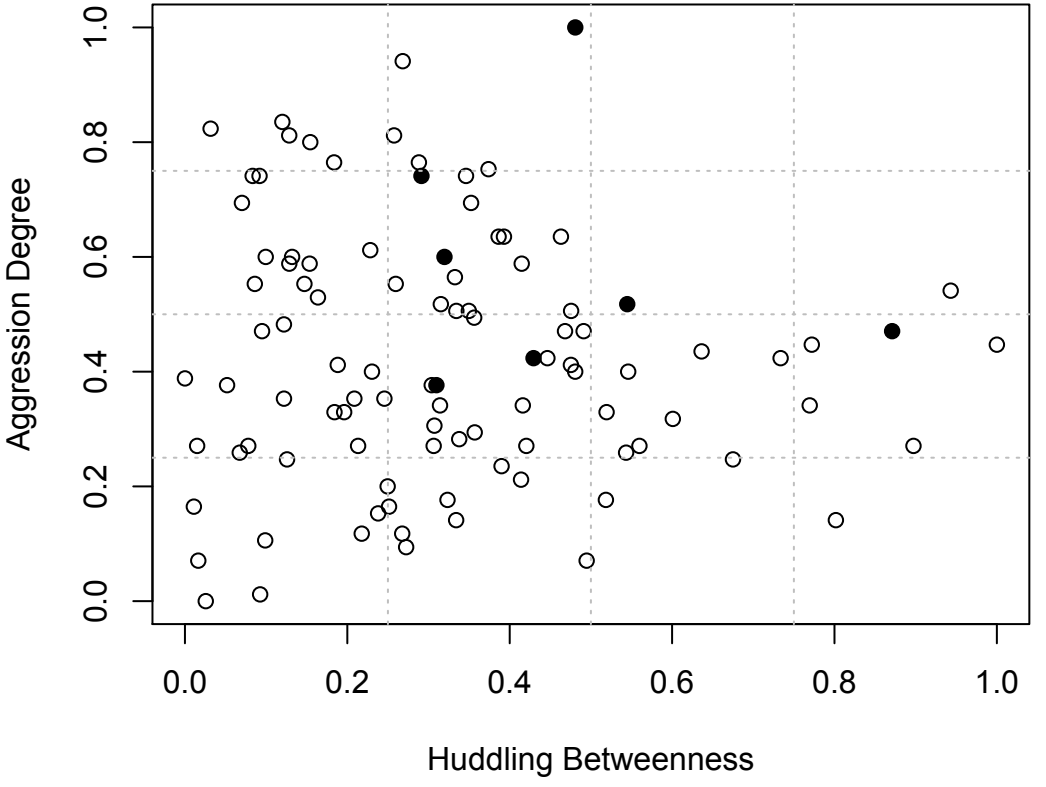

Table 1(on next page)

Summary of model statistics and parameter estimates from the *candidate model set* (all models with a ΔAIC < 2; outcome: Shigella presence-absence) for rhesus macaques in Groups I & II

Model Number	Predictor(s)	df	β	Adj. SE	P	AIC	AICc	Δ	w
7	Intercept	195	0.83	0.39	0.035*	169.37	169.43	0	1
	Groom Eigenvector		-2.76	1.22	0.023*				
20	Intercept	193	-0.02	0.70	0.98	170.14	170.34	0.92	0.67
	Huddle Betweenness		-8.60	3.34	0.01*				
	Dominance Certainty		-2.48	1.43	0.08(*)				
	Huddle Betweenness : Dominance Certainty		13.55	6.06	0.026*				
4	Intercept	195	-0.91	0.41	0.026*	171	171.06	1.63	0.42
	Groom Outdegree		-2.31	1.16	0.047*				
9	Intercept	195	-1.14	0.32	0.001*	171.2	171.26	1.84	042
	Huddle Betweenness		-2.38	1.27	0.06(*)				

***** **p < 0.05;**
****** **p < 0.01**
(*) **0.05 < p < 0.1**
Δ: Difference in AICc score from the best-fit model
w: Relative model weight

Table 2(on next page)

Summary of model statistics and parameter estimates from the *candidate model set* (all models with a ΔAIC < 2; outcome: Shigella presence-absence) for rhesus macaques in Group III

Model Number	Predictor(s)	df	β	Adj. SE	P	AIC	AICc	Δ	w
44	Intercept	97	-5.47	1.33	0.001**	45.27	45.52	0	1
	Aggression Outstrength		5.09	1.81	0.005**				
	Huddle Betweenness		3.42	1.87	0.067(*)				
42	Intercept	97	-5.97	1.52	0.001**	46.17	46.42	0.9	0.62
	Aggression Outdegree		5.12	1.95	0.008**				
	Huddle Betweenness		3.31	1.84	0.072(*)				
13	Intercept	98	-3.93	0.73	0.001**	46.52	46.65	1.13	0.56
	Aggression Outstrength		4.56	1.61	0.004**				
11	Intercept	98	-4.28	0.93	0.001**	47.30	47.43	1.91	0.38
	Aggression Outdegree		4.53	1.72	0.008**				

*** $p < 0.05$;**
**** $p < 0.01$**
(*) $0.05 < p < 0.1$
Δ: Difference in AICc score from the best-fit model
w: Relative model weight