



Original Article

Physiological and social consequences of gastrointestinal nematode infection in a nonhuman primate

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Gastrointestinal nematodes are intensely studied models for host–pathogen interactions in wildlife, yet consequences of infections are not fully understood. Among the potential costs of nematode infection are physiological changes caused by immune system activation, reduction or reallocation of available energy, as well as potential social consequences in terms of decreased social activity or avoidance of infected individuals. We used experimental anthelmintic treatment to investigate effects of **strongyle nematode infection** in Barbary macaques (*Macaca sylvanus*), comparing 56 treated to 17 untreated individuals. **Deworming success was monitored by coproscopy and infection probability estimated from patch occupancy models.** Increasing strongyle infection probabilities were associated with **increased fecal glucocorticoid metabolite levels and slightly decreased activity** and had no significant effect on energy balance quantified as urinary C-Peptide levels. The frequency to approach into close spatial proximity of a partner was predicted by the partner's, but not focal individual's infection status, with a tendency toward infected individuals being approached less frequently. Although effects were weak, they suggest a co-occurrence of sickness behavior and avoidance of infected conspecifics, both possibly shaping social interaction patterns with potential consequences for an individual's social relationships. This study adds to the growing body of research on the complex interactions of sociality, health, and fitness in a group living species.

Key words: avoidance behavior, parasites, physiology, primates, sickness behavior.

INTRODUCTION

Gastrointestinal (GI) nematodes are studied in wildlife for their impact on host behavior, health, and evolution (Ezenwa 2004a; Martin et al. 2011; Tompkins et al. 2011). **For GI nematodes with direct life cycles, eggs are shed in feces;** infectious stages develop in the environment and infections occur on contact with or ingestions of infectious stages, for example, via contaminated soil or food

(Anderson 2000; Bethony et al. 2006). Thus, parasite transmission typically occurs environmentally—although the possibility of direct transmission via grooming has been suggested (Hernandez and Sukhdeo 1995; MacIntosh et al. 2012)—and is linked to host behavior via exposure to infectious stages (Altizer et al. 2003; Hawley et al. 2011). Frequent social contact and high social integration are linked with high GI nematode infection probability (Fenner et al. 2011; Rimbach et al. 2015; Wren et al. 2016; Friant et al. 2016b), suggesting that social interactions contribute to GI nematode transmission; to which extent transmission results from either shared use of contaminated space (MacIntosh et al. 2012;

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Rimbach et al. 2015; Friant et al. 2016b) or direct interactions (Wren et al. 2016) is still under debate. As GI parasite infections often simultaneously influence host behaviors (Ghai et al. 2015; Chapman et al. 2016; Ezenwa et al. 2016), GI nematodes can be excellent models for evolutionary feedback loops and are proposed drivers of social evolution (Freeland 1976; Chapman et al. 2009). Yet, causes and effects of infections are often not well understood in wildlife due to the correlational nature of most studies.

GI parasite infections often appear subclinical (see) have been described to impact host physiology and health, sometimes dramatically. Infection-related tissue damage, inflammation, and immune responses (Pit et al. 2001; Andreasen et al. 2016) can be energetically costly (Derting and Compton 2003; Colditz 2008). As infections with GI nematodes can lead to impaired food absorption (Coop and Holmes 1996), parasites are commonly associated with poorer physical condition and nutritional status in several animal taxa (Stien et al. 2002; Díaz and Alonso 2003; Ezenwa 2004b; Irvine et al. 2006). In humans, consequences of severe infections, like malnutrition and anemia, can result in stunted growth and impaired cognitive development (Stephenson et al. 2000a; Stephenson et al. 2000b). In wildlife, the health impact of GI nematodes is less clear, as individuals often do not show overt symptoms (Krief et al. 2008).

Sickness behavior, a general response to infection mediated by inflammatory cytokines (Dantzer 2001; Kongsman et al. 2002), is one host strategy to limit the costs of infections. Indicators of sickness behavior are lethargy and heat-conserving body posture, both aiming at preserving energy and behaviorally increasing body temperature to support physiological fever (Hart 1988; Kyriazakis et al. 1998; Kongsman et al. 2002; Dantzer 2004). Lower activity levels and reduced behavioral flexibility have been linked to GI nematode infections in primates (MacIntosh et al. 2011; Ghai et al. 2015; Chapman et al. 2016; Friant et al. 2016a), implying sickness behavior in response to infections. Decreased food intake, a further characteristic of sickness behavior, can contribute to heat conservation via reduced movement, and promotes immune efficiency (Kongsman et al. 2002). Together, these behavioral changes are considered as adaptive responses to infection, increasing survival (Hart 1988; Kyriazakis et al. 1998; Kongsman et al. 2002; Dantzer 2004).

The inflammatory processes inducing sickness behavior also lead to the release of glucocorticoids (GCs), which is vital for immune response regulation (Besedovsky et al. 1986; Kongsman et al. 2002). As poorly regulated immune responses can cause immunopathology (Graham et al. 2005) and immune responses can themselves be costly (Hart 1988; Bonneaud et al. 2003; Derting and Compton 2003; Colditz 2008), these regulatory effects of GCs are crucial. Another role of GCs is contributing to parasite tolerance, that is, managing the damage caused by infection rather than mounting immune responses aimed at parasite clearance in order to minimize infection costs (Read et al. 2008; Råberg et al. 2009; Medzhitov et al. 2012; Råberg 2014). GC levels are among the most commonly studied physiological markers in relation to GI nematode infections and a wealth of studies report infections being associated with higher GC levels (e.g. Fleming 1997; Chapman et al. 2007; Pedersen and Greives 2008).

Infections with GI nematodes not only impact host physiology but also host social behavior, with infected individuals being less socially active (Chapman et al. 2016; Friant et al. 2016a). This decreased social activity can be attributed to sickness responses or avoidance of energetically costly behaviors by infected individuals. Another, nonmutually exclusive explanation is avoidance of infected

individuals by conspecifics (Chapman et al. 2016; Friant et al. 2016), as demonstrated in mandrills (*Mandrillus sphinx*), which avoid grooming individuals infected with directly transmitted unicellular GI parasites (Poirotte et al. 2017). For investigating the directionality of the host-parasite interactions regarding both physiology and behavior in wildlife (Hawley and Altizer 2011; Martin et al. 2011), experimental studies, which are still relatively rare to date (Ezenwa et al. 2010; Pedersen and Fenton 2015; Chapman et al. 2016; Friant et al. 2016b; Poirotte et al. 2017), will be imperative.

Here, we capitalized on successful strongyle nematode removal by routine anthelmintic treatment of two groups of semi free-ranging Barbary macaques (*Macaca sylvanus*) to investigate the relationship between GI parasite infection, physiology, and social behavior. We assessed how infection status relates to fecal GC metabolite (fGCM) levels, predicting decreased GC levels in treated individuals. To test whether infections impact host physical condition, we measured levels of urinary C-Peptide (uCP), a validated marker of energy availability in primates (Deschner et al. 2008; Emery Thompson and Knott 2008; Higham et al. 2011), predicting increased uCP levels in treated individuals. We tested whether GI nematodes impact host activity, predicting lower activity, a common sign of sickness behavior, in infected individuals. We also investigated social consequences of infections, aiming at assessing whether changes in social behavior were driven by sickness behavior (changes depending on own infection status) or avoidance of infected individuals (changes depending on partner infection status). To this end, we analyzed patterns of directed approaches into and departures from close proximity on a dyadic level, taking infection status of both interaction partners into account.

MATERIALS AND METHODS

Study site, subjects, and design

The study was conducted at Affenberg Salem, a 20-ha forested outdoor enclosure in Germany, housing three freely interacting groups of Barbary macaques (de Turkheim and Merz 1984). We collected behavioral data and samples on all adult individuals (females > 4 years, males > 6 years) of two groups ($n = 77$, 4 excluded from analyses, see below) with similar age-sex compositions, between June and December 2014 and 2015 (Table 1). For anthelmintic treatment, food items containing ivermectin (Diapec®, Albrecht, Aulendorf, Germany), a broad spectrum anthelmintic compound, were individually administered on a single day in August under supervision of the population veterinarian, following standard study site protocols (dosage ~0.4-mg/kg body weight). In 2014, all individuals were treated. In 2015, 20 individuals served as untreated controls, with this control group being matched for sex, age, male immigration status (natal vs. immigrant), and female matriline to the treatment group (Table 1). For population health management reasons, all individuals were treated in November 2015. Collection of behavioral data was terminated on this second treatment in 2015, whereas sample collection continued until the end of the study. Consequently, physiological analyses included the effect of both treatments in 2015, but behavioral analyses include only the effect of the first treatment in both study years.

Behavioral data collection

Behavioral data on affiliative and agonistic interactions were collected by 40 and 45 min continuous focal animal sampling (Martin and Bateson 2007) from June to December 2014 and June to November

Table 1
Overview over the study subjects and design. All adult individuals were included in the study

Year	2014, group C	2015, group H
Study group	16 males (age 6 to 28 years) 20 females (age 4 to 27 years) 23 immatures	18 males (age 6 to 25 years) 23 females (age 5 to 29 years) 19 immatures
Treatment group composition	All individuals	9 males, age 6 to 25 years (14.5 ± 6.5 years) 12 females, age 6 to 26 years (14.3 ± 6.8 years)
Control group composition	None	9 males, age 6 to 24 years (14.2 ± 6.9 years) 11 females, age 5 to 29 (14.2 ± 7.4 years)
Sample collection	4th June–1st Dec 14	7th June–30th Nov 15
Data collection	16th June–1st Dec 14	16th June–31st Oct 15
Treatment	5th Aug 14, all individuals	17th Aug 15, treatment group 1st Nov 15, all individuals
Phases for social behavior analyses		
Pre-phase	24th June–4th Aug 14	6th July–16th Aug 15
Post-1-phase	6th Aug–16th Sep 14	18th Aug–28th Sep 15
Post-2-phase	17th Sep–28th Oct 14	29th Sep–31st Oct 15
Post-3-phase	29th Oct–1st Dec 14	NA

2015 (3245.6 observation hours, 12.2 ± 2.9 h/individual/phase; 48.1 ± 4.6 h/individual in 2014, 36.9 ± 1.4 h/individual in 2015). During focal animal protocols, individual activity and position were recorded each minute using instantaneous recording (Martin and Bateson 2007) ($n = 201\,784$ instantaneous records). Agonistic interactions were additionally recorded *ad libitum* (Martin and Bateson 2007) and all decided dyadic interactions (clear submission from only one individual, no counter aggression; $n = 3952$ in 2014, $n = 4582$ in 2015) were used to determine individual dominance rank (CombiCalc score) using DomiCalc (Schmid and De Vries 2013).

Social behavior parameters

Due to the non-normal distribution of data, we could not use durations obtained by focal animal sampling to assess the impact of infections on behavior. Instead, we used the number of directed dyadic approaches as a proxy of time spent in proximity to a partner, which were strongly correlated with time spent in proximity (row-wise matrix correlation, individual row-wise Kendall's tau min 0.675, max 0.865). We assessed whether treatment affected approach numbers based on individual or partner infection status. Similarly, we used directed departures from close proximity to test whether avoidance of infected individuals could be facilitated by frequent departures from infected conspecifics. As grooming patterns were highly differentiated and most dyads were never observed grooming (1261 of 1298 dyads), we focused our analyses on approaches and departures.

To achieve the best compromise between temporal resolution of analyses and the representativeness of social behavior patterns, we split the study period into four 6-week phases (one pre-phase prior to first treatment and three phases following treatment in 6-week increments, see Table 1) with roughly equal observation times per dyad (24.5 ± 4.2 focal h/dyad) for statistical analysis. The 6-week period was chosen because firstly, egg shedding variability was still reasonably high within 6 weeks of treatment (see below). Secondly, and more importantly, after 6 weeks, the number of interaction partners began to stabilize (see Supplementary Figures F1 and F2 for visualization of cumulative approach rates and # of interaction partners over weeks of observation), indicating that interactions between more rarely interacting dyads were sufficiently represented in the data.

We calculated dyadic composite sociality indices (CSI) (Silk et al. 2006) for the entire study period as a measure of social relationship strength to use as a control variable in social behavior analyses. We included both duration (min/focal hour) and frequency of proximity within 1.5 m, body contact, and grooming (Young et al. 2014), using the following formula:
$$\frac{\left(\frac{FF_{ij}}{F_{iav}}\right) + \left(\frac{DF_{ij}}{D_{iav}}\right) + \left(\frac{FB_{ij}}{F_{iav}}\right) + \left(\frac{DB_{ij}}{D_{iav}}\right) + \left(\frac{FG_{ij}}{F_{iav}}\right) + \left(\frac{DG_{ij}}{D_{iav}}\right)}{6}$$
 with $ij = \text{dyad}$, $av = \text{group mean}$, $F = \text{frequency}$, $D = \text{duration}$, $P = \text{proximity}$, $B = \text{body contact}$, and $G = \text{grooming}$. As the sexes differed markedly in affiliation rates, we calculated same and opposite sex dyadic CSIs separately. The six measures were all positively correlated in row-wise matrix correlations (average row-wise tau 0.25–0.94). Based on average interaction rates and distribution of interactions in our study population, constructing CSIs for shorter time periods was not feasible.

Sample collection

We aimed at collecting weekly fecal samples for the entire study period. Samples were collected immediately after defecation from all focal individuals for parasite analysis, and a separate aliquot of the homogenized feces was stored for fGCM analysis. If size of the fecal matter was small, priority was given to parasite samples; a subsequent second sample for fGCM analyses was collected as soon as possible. For measurement of uCP levels, we opportunistically collected urine samples without fecal contamination from focal individuals, using clean plastic sheets and salivettes (Salivette® Cortisol, Sarstedt, Nürmbrecht, Germany) (Danish et al. 2015; Müller et al. 2017). On collection, all samples were stored in a thermos flask containing ice until further processing (recovery of urine from salivettes and fixing samples for parasite analyses with 10% formalin) within 12 h of sample collection (Danish et al. 2015; Müller et al. 2017). Parasite samples were stored in the dark at room temperature until examination at the Institute for Parasitology of the University of Veterinary Medicine Hannover, Germany, fGCM, and uCP samples were frozen at –20 °C until analysis at the Endocrinology Laboratory of the German Primate Centre.

Hormone analyses

For fGCM analysis, fecal samples ($n = 1227$; $n = 519$, 14.8 ± 1.6 samples/individual, i.e. 3.7 ± 0.8 samples/individual/phase in 2014, $n = 708$, 18.6 ± 1.2 samples/individual, i.e. 4.7 ± 1.3

samples/ individual/phase in 2015) were lyophilized, pulverized and subsequently extracted with 3 mL of 80% watery methanol (Palme et al. 2013) as described in detail by Heistermann et al. (1995). We analyzed fecal extracts for concentrations of cortisol metabolites using an enzyme immunoassay for the measurement of immunoreactive 11 β -hydroxyetiocholanolone (Ganswindt et al. 2003), previously validated for assessing GC output in numerous primate species, including Barbary macaques (Heistermann et al. 2006). Prior to hormone measurement, fecal extracts were diluted 1:80 in assay buffer and duplicate 50 μ L aliquots of diluted samples were taken to assay, which was carried out as described in Heistermann et al. (2004). Sensitivity of the assay was 0.6 pg/well. Intra- and interassay coefficients of variation (CVs), calculated from replicate measurements of high- and low-value quality controls, were 5.9% (high, $n = 16$) and 7.9% (low, $n = 16$) and 7.5% (high, $n = 80$) and 9.1% (low, $n = 80$), respectively. All fGCM concentrations are expressed as ng/g fecal dry weight.

Urine samples were available for 60 individuals. We measured uCP levels in duplicates ($n = 400$; $n = 52$, 2.4 ± 1.5 samples/individual, i.e. 1.4 ± 0.5 samples/individual/phase in 2014, $n = 348$, $n = 8.9 \pm 2.4$ samples/individual, i.e. 2.4 ± 0.8 samples/individual/phase in 2015) as described in Müller et al. (2017). Assay sensitivity was 0.064 ng/mL. Intra-assay CVs, determined as described for fGCM measurements (see above), were 5.2% (high, $n = 18$) and 8.8% (low, $n = 18$), respectively, although values for interassay CVs were 7.0% (high, $n = 22$) and 14.2% (low, $n = 22$). uCP concentrations were indexed by the sample's specific gravity (Emery Thompson et al. 2012), using the formula given in Miller et al. (2004): $SG - corrected\ value = raw\ value \times \frac{(SG_{mean} - 1.0)}{(SG_{sample} - 1.0)}$.

Parasite analysis

If available, we analyzed weekly individual samples and every available sample in the first 2 weeks after treatment to assess deworming efficiency ($n = 1436$, 18.6 ± 3.5 samples/individual; $n = 567$, 15.8 ± 3.0 samples/individual, i.e. 4.0 ± 1.6 samples/individual/phase in 2014, $n = 869$, 21.2 ± 1.1 samples/ individual, i.e. 5.3 ± 1.6 samples/individual/phase in 2015) using the McMaster method as described in Müller et al. (2017). Three morphotypes, strongyle nematodes, *Capillaria* spp., and *Trichuris* spp. were detected. There was no evidence for the presence of further GI parasite taxa (e.g. unicellular parasites, tapeworms). Individuals stopped shedding strongyle nematode eggs within 2 days of treatment and did not resume shedding for at least 3 weeks (Müller et al. 2017, see Supplementary Figure F3), but some individuals continued to shed *Capillaria* and *Trichuris* eggs (see Supplementary Figures F4 and F5). As this indicates successful clearance of strongyle nematodes, but not *Capillaria* and *Trichuris* (Müller et al. 2017), predictions were tested for strongyle nematodes only. Due to the extremely low number of *Trichuris* positive samples ($n = 25$ samples from 10 individuals), we did not include *Trichuris* in further analyses. *Capillaria* coinfection was considered if including it significantly improved model fit (model comparison using a likelihood ratio test with the R function Anova, setting the argument test to "Chisq," $P < 0.05$).

Patch occupancy modeling

Coprospectical parasite analysis has some inherent methodological constraints, resulting from detection sensitivity and inconsistencies in egg shedding, which can lead to nondetection of infections. Additionally, egg counts not necessarily reflect worm burden and

thus infection intensity (Christensen et al. 1995; Roepstorff et al. 1996), leading many to dismiss egg counts in studies of individual infection (Gillespie 2006). In this study, the egg shedding intensity for strongyle nematodes was rather low; in the majority of samples positive for strongyle nematodes ($n = 277$ of 514 positive samples), egg counts equaled the detection sensitivity of 25 egg per gram of feces. Therefore, the likelihood of not detecting eggs in feces of de facto strongyle positive individuals was high (Figure 1).

We estimated the likelihood of detection of infection in a given week from those cases where several samples from the same individual and week were available. Rather than manually assigning probable infection states to an individual, we used parasite morphotype specific colonization-detection-extinction patch occupancy models, using the "unmarked" package (version 0.2–12), in R (Fiske and Chandler 2011). This step was taken to mitigate issues with low detection rate, to account for missing data ($n = 755$ individual weeks) and to obtain a more accurate estimate of individual parasite status. In contrast to using egg count data (which leads to an underestimation of parasite infections due to low egg shedding intensity in this study), patch occupancy considers individuals positive if detection occurs once within a week and estimates the likelihood of infection based on detection, extinction, and colonization probabilities within the egg count dataset.

For patch occupancy modeling, we considered each individual as one patch, assumed a maximum of four observation events per week and coded parasite egg presence or absence as a binary variable. Samples of individuals shedding strongyle eggs within 2 days of treatment ($n = 10$ samples of 9 individuals) were excluded, as these were probably attributable to the gut passage of eggs remaining after parasite clearance. The models assumed a constant detection and initial egg presence probability. Colonization probability (i.e. changing from no eggs present in week x to eggs present in week $x + 1$) was allowed to vary between individuals by including ID as a factor, modeling possible inter-individual differences in colonization. Extinction probability (i.e. changing from eggs present in week x to eggs absent in week $x + 1$) was modeled by the effect of treatment (treatment = yes in week x influencing clearance probability for week $x + 1$). Overall strongyle extinction probability was 4.1% but increased to 100% after treatment, indicating successful clearance. We extracted the estimated egg presence probability ranging from 0 to 1, hereafter referred to as infection probability, for the respective individual and week. Infection probability patterns closely resembled egg shedding patterns obtained by coproscopy (Figure 1).

In the end, we used weekly infection probabilities as measures of parasite status in all subsequent statistical analyses as a more cautious representation of the individual infection status and a way to also capture measurement uncertainty. Because strongyle infection probability was directly predicted by treatment in patch occupancy modeling, experimental group as a control variable could not be included in linear models due to collinearity issues. To better maintain the experimental design of the study, we excluded three untreated individuals which showed spontaneous parasite clearance from further analyses (see Figure 1a), so treatment is modeled indirectly by infection probability. We also excluded one female which died in September after a period of lethargy and diarrhea (cause of death could not be determined as no necropsy could be performed), as her condition might have influenced her physiology and behavior, so all statistical analyses were performed on data of the remaining 73 individuals.

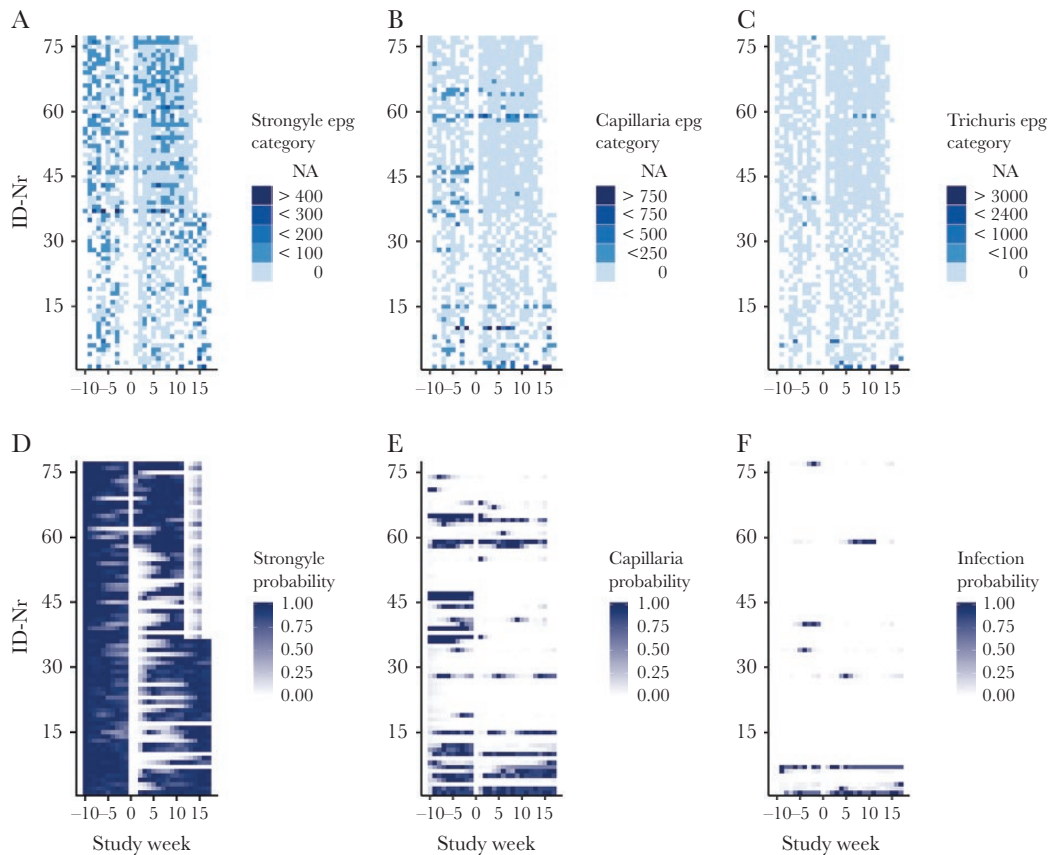


Figure 1

Fecal egg counts (eggs per gram = epg) (A through C) and corresponding infection probabilities estimated by patch occupancy modeling (D through F) for strongyle nematodes, *Capillaria* spp. and *Trichuris* spp. Individuals 1–36 belong to the study group of 2014, individuals 37–77 to the study group from 2015. Individuals 1–57 were treated (week 0), individuals 58–77 represent the control group, individuals 37–77 were treated again between week 11 and 12. Within each year and experimental group, individuals are arranged by age, increasing from the top. Egg counts were summarized into the categories displayed in the legends for each morphotype due to the low variability. Missing samples are indicated by white fields (A through C).

Statistical analyses

To test whether strongyle nematode infections increase fGCM and decrease uCP levels, we ran linear mixed models (LMMs) with log-transformed fGCM and uCP levels as response, strongyle probability as the main predictor and individual ID as random factor. *Capillaria* infection probability was included as a main effect in both models, as it improved model fit. Individual sex, rank, age, and social group were included as control variables. **In seasonal breeders like Barbary macaques, seasonal physiological changes can be sex-specific.** To account for this and differences between the study years, we included study week as a predictor and tested whether including a nonlinear effect of study week (fitted as a quadratic term), an interaction between sex and study week, and an interaction between study year and week significantly improved the model fit. The best models included a nonlinear effect of study week for both models and an interaction between study week and sex for the fGCM model. All linear predictors were z -transformed prior to analysis.

Activity

To assess the effect of strongyle infection on activity, positional data from instantaneous recordings were used, defining sitting and lying as inactive and all other positions (e.g. running, walking, climbing) as active. Activity was analyzed on a weekly basis using an n/k

binomial generalized LMM (GLMM), with n = number of active instantaneous records and k = number of inactive instantaneous records for each individual as response. Strongyle infection probability of the corresponding week was the main predictor; individual sex, rank, age, the interaction between study year and quadratic effect of week (modeling study year specific changes in activity patterns), and *Capillaria* infection probability were included as control variables, individual ID as random factor. Linear predictors were z -transformed prior to analysis.

Social behavior

To assess the effect of strongyle infection probability on social behavior, we ran zero-inflated negative binomial GLMMs with number of directed approaches to and departures from a specific partner as response (i.e. the number of approaches focal animal A directed to partner B in the respective phases pre-treatment, post-treatment 1, post-treatment 2, and post-treatment 3), controlling for sampling effort by including log-transformed focal observation hours as an offset term. Average strongyle infection probabilities of each interaction partner for the corresponding phase were used as the two main predictors to distinguish between effects of sickness behavior and avoidance. Individual sex, age, rank, dyadic CSI, study year, and study phase were included as control variables, focal animal and partner ID were included as random factors.

For the departure model, we tested whether model fit was improved by including interaction terms between individual infection probability and number of approaches directed toward a partner and between partner infection probability and number of partner approaches. The interactions were included to test whether individuals are less likely to depart from a partner if infected themselves or are more likely to depart from infected partners that approached them often. Only the interaction between individual infection probability and approach numbers was retained, as it significantly improved the model fit. All linear predictors were z -transformed prior to analysis.

All analyses were carried out in R, version 3.5.1 (R Core Team 2014) using the `glmmadmb` package, version 0.8.3.3 (Bolker et al. 2011). For all models, the reference level of sex was female, for study year 2014. Effect sizes (conditional R^2 values for the whole model) were obtained by dividing the explained variance (variance of fixed and random effects) by the total variance (explained and residual variance). For each model, various model diagnostics were employed to confirm model validity (visual inspection of distribution of residuals, qq plots, residuals plotted against fitted values, variance inflation factors using the package “car” for LMMs, and absence of overdispersion for negative binomial models) none of which suggested violation of model assumptions.

Ethical statement

This study was conducted noninvasively and adhered to the Animal Behavior Society’s Guidelines for the treatment of animals in behavioral research and teaching (2012) and to standards on the protection of animals used for scientific purposes as defined by the European Union Council Directive 2010/63/EU. Authorization for the anthelmintic treatment was given by the Veterinary Office of the district office of county Lake Constance, and treatment was performed as defined by the European Union Council Directive 1999/22/EC as part of the routine procedures on Affenberg Salem. The study was approved by the Animal Welfare Body of the German Primate Centre (No. E9-16).

RESULTS

Parasite analysis and efficacy of anthelmintic treatment

We identified three egg morphotypes, strongyle nematodes (100% prevalence), *Capillaria* spp. (42.9% prevalence), and *Trichuris* spp.

(13.0% prevalence). After treatment, strongyle nematode egg shedding of treated individuals stopped within 2 days and did not resume until 3 weeks after treatment. For both *Capillaria* and *Trichuris*, egg shedding continued after treatment in 9 and 5 treated individuals, respectively, suggesting that anthelmintic treatment was not fully effective against these morphotypes (Supplementary Figures F3–F5). Strongyle nematodes are difficult to identify on a genus level based on egg morphology, yet based on data available from past necropsies (Roland Hilgartner, pers. communication) and preliminary results from larval cultures (NMK, unpublished data), the most common strongyle nematode in the study population is *Oesophagostomum* spp., although the presence of hookworms or *Trichostrongylus* spp. cannot be excluded.

Impact of parasite infection on physiology and activity

Physiological parameters and activity were highly variable both within and between individuals. Fecal GCM was similar between both study years and experimental groups, whereas uCP levels and activity differed between the study years but were comparable between both experimental groups in 2015 (Table 2). Levels of fGCM significantly increased with individual age, dominance rank, and with progressing time into the study in males but not females (Table 3, Figure 2a). Controlling for these effects, strongyle

Table 2

Overview over physiological data and activity patterns of 77 semi free-ranging Barbary macaques, categorized by study year and experimental group

	fGCMs (ng/g feces)	uCP (ng/mL corr. specific gravity)	% active
Overall			
Range	21.1–1980.7	0.05–210.6	0.00–0.87
Mean \pm SD	383.3 \pm 222.2	10.3 \pm 21.0	0.19 \pm 0.14
group C			
Range	21.1–1870.6	0.3–20.0	0.00–0.87
Mean \pm SD	381.5 \pm 199.6	3.0 \pm 3.4	0.24 \pm 0.16
group H treatment			
Range	75.0–1912.6	0.1–210.6	0–0.52
Mean \pm SD	393.5 \pm 255.8	11.2 \pm 24.0	0.14 \pm 0.10
group H control			
Range	69.1–1980.7	0.05–178.6	0–0.52
Mean \pm SD	373.3 \pm 211.5	11.6 \pm 20.0	0.13 \pm 0.08

Table 3

Results from LMM analysis of the effect of strongyle infection probability on log-transformed fGCM levels, controlling for the effects of age, sex, rank, study year, and a quadratic term of study week ($n = 1227$ samples on 73 individuals). We controlled for a seasonal effect in males, but not females, by including an interaction term between sex and study week. Individual ID was included as a random factor and all linear predictors z -transformed prior to analyses

	Estimate	SE	95% confidence intervals		z	P
Intercept	5.708	0.057	5.597	5.820	100.441	<0.001
Age	0.084	0.028	0.029	0.139	3.003	0.003
Sex	0.087	0.097	−0.104	0.278	0.895	0.371
Rank	−0.130	0.048	−0.224	−0.035	−2.688	0.007
Study year	0.041	0.054	−0.066	0.147	0.745	0.456
Week	0.005	0.015	−0.024	0.035	0.349	0.727
Week ²	0.058	0.012	0.034	0.081	4.717	<0.001
Sex \times week	0.160	0.022	0.117	0.203	7.272	<0.001
<i>Capillaria</i> probability	−0.035	0.017	−0.067	−0.002	−2.098	0.036
<i>Strongyle</i> probability	0.033	0.013	0.008	0.058	2.578	0.010

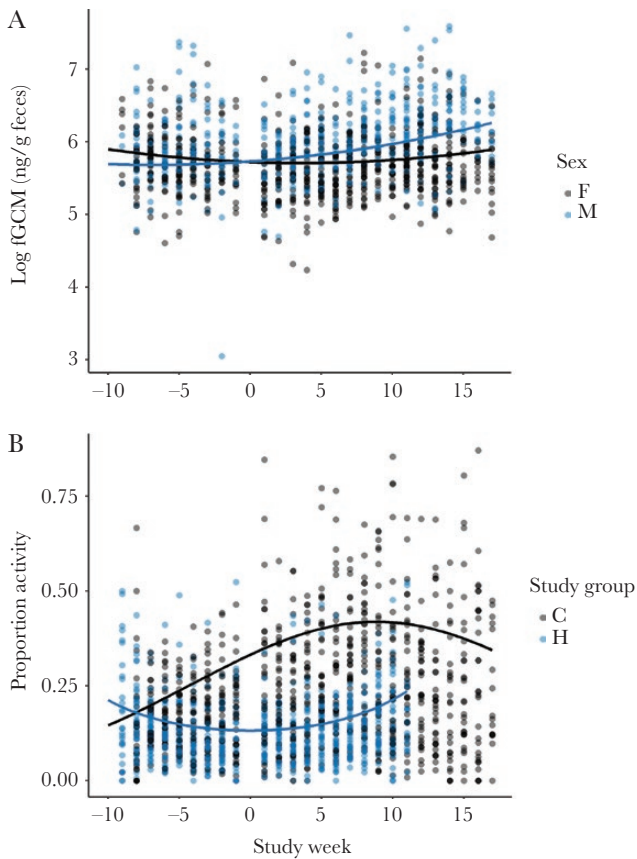


Figure 2

Seasonal variation in (A) fGCM level and (B) activity. Each datapoint represents one individual samples (A) or the activity of one individual in the corresponding study week. Seasonal differences were detected between the sexes for fGCM levels and between both study years (group C in 2014 and H in 2015), respectively. The effects of strongyle infection on physiology and behavior were analysed controlling for the temporal effects illustrated here.

infection probability was positively correlated to fGCM levels (estimate = 0.033 ± 0.013 , $z = 2.578$, $P = 0.010$, Table 3, Figure 3a), with the model explaining ~41% of the variance in fGCMs (conditional $R^2 = 0.411$). The effect of changing from infection probability 0 to 1 leads to a fGCM increase equivalent of aging 11 years. Coinfection with *Capillaria* did not modulate the strongyle effect on fGCM (interaction term strongyle and *Capillaria* infection probability did not improve model fit) but controlling for strongyle infection, *Capillaria* infection probability had a negative effect on fGCM levels (estimate = -0.035 ± 0.017 , $z = -2.098$, $P = 0.036$).

Levels of uCP showed a seasonal pattern, with lower values in summer. The only significant predictor of uCP levels was age, with older individuals having significantly lower levels (estimate = -0.238 ± 0.110 , $z = -2.169$, $P = 0.030$, Table 4). Strongyle infection probability was not significantly related to uCP levels (estimate = -0.049 ± 0.094 , $z = -0.524$, $P = 0.600$, Table 4, Figure 3b), and the effect of *Capillaria* infection probability was only a statistical trend (estimate = -0.167 ± 0.100 , $z = -1.664$, $P = 0.096$). Overall, the model explained only ~13% of the uCP variability ($R^2 = 0.131$).

Activity followed significantly different seasonal patterns in both study years (interaction week² × study year: estimate = $0.411 \pm$

0.019 , $z = -22.000$, $P < 0.001$, Table 5, Figure 2b) and was significantly lower in older individuals and males (Table 5). Controlling for these effects, activity showed a trend to decrease with increasing strongyle infection probability (estimate = -0.015 ± 0.009 , $z = -1.728$, $P = 0.084$, Table 5, Figure 3c) and was significantly negatively correlated to *Capillaria* infection probability (estimate = -0.021 ± 0.010 , $z = -2.058$, $P = 0.040$). The effects of *Capillaria* were stronger, with changing from strongyle or *Capillaria* probability from 0 to 1 equaling an activity reduction of aging ~2 and 6 years, respectively. The model explained ~28% of variance in activity ($R^2 = 0.282$).

Impact of parasite infection on social behavior

The number of times individuals of a dyad approached each other was low for most dyads, and ~60% of dyads were never observed approaching each other throughout the entire study period. Behavioral patterns were similar between the study phases and experimental groups but differed between the study years (Table 6). The number of approaches and departures per dyad were significantly influenced by individual age, sex, rank, and dyadic CSI (Tables 7 and 8) and were higher in 2014 than in 2015. We also found a significant impact of study phase on approaches and departures (Tables 7 and 8). Controlling for these effects, individuals tended to reduce their approach behavior the higher the infection probability of the partner was (estimate = -0.058 ± 0.030 , $z = -1.955$, $P = 0.051$, Table 7), which translated into a ~15% reduction of approaches directed to infected compared to uninfected partners. The overall model explained ~59% of approach variance ($R^2 = 0.587$).

The number of departures from close proximity was not linked to partner infection probability (estimate = -0.009 ± 0.024 , $z = -0.378$, $P = 0.705$, Table 8), but we found a significant interaction effect between the actor's infection probability and number of approaches (estimate = -0.036 ± 0.015 , $z = -2.337$, $P = 0.019$, Table 8, Figure 4). Although individuals generally departed more frequently from partners they approached more often (main effect of approaches to partner: estimate = 0.441 ± 0.018 , $z = 23.938$, $P < 0.001$), this effect was reversed when individuals were likely infected with strongyles so that individuals with high infection probabilities departed individuals they often approached less. Independent of these effects, we also observed the more departures the more often the focal was approached by a partner (estimate = 0.609 ± 0.020 , $z = 30.535$, $P = 0.009$), irrespective of partner infection status (interaction partner approaches × partner infection probability did not significantly improve model fit). The overall variance explained by the model was ~56% ($R^2 = 0.564$).

DISCUSSION

We investigated the effects of strongyle nematode infections in Barbary macaques using anthelmintic treatment. Infections were ubiquitous in our study population and *Oesophagostomum spp.*, a parasite commonly found in nonhuman primates (Gillespie et al. 2004; Kooriyama et al. 2010; Krief et al. 2010; Ghai et al. 2014; Terio et al. 2016) was the most important strongyle nematode present. Strongyle infections in general and *Oesophagostomum* infections in particular often do not cause overt symptoms (Krief et al. 2008). As *Oesophagostomum* stage 3 larvae cause lesion in the intestinal wall during the cause of infections—which can cause severe illness (Krief et al. 2008; Terio et al. 2016)—infections are nonetheless

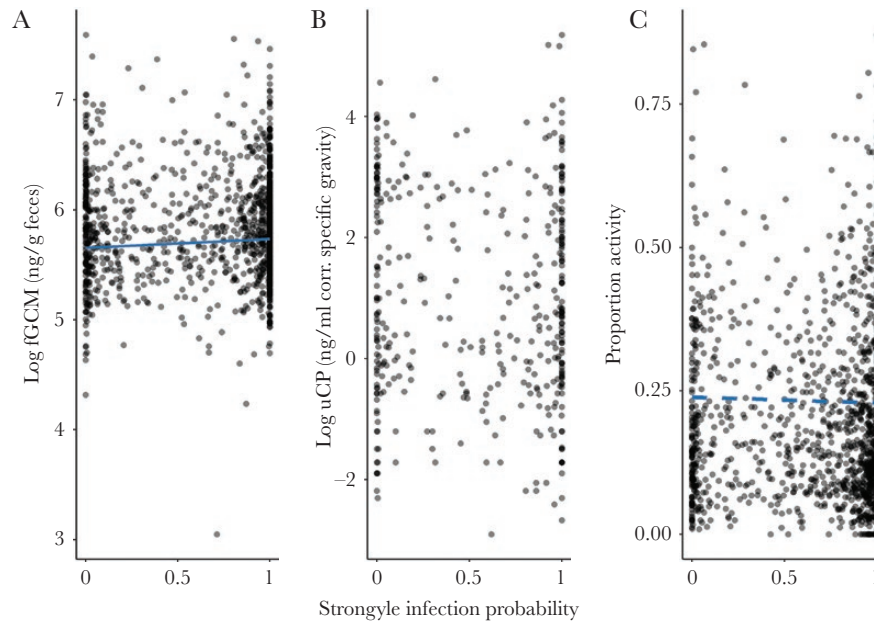


Figure 3

Effect of strongyle infection probability on (A) fGCM, (B) uCP and (C) activity. Each datapoint represents one individual sample (A, B) or the activity of one individual in the corresponding study week. Regression lines show the effect on fGCMs and activity, respectively, accounting for the average effects of week, sex, and study group. We found no significant effect of infection probability on uCP levels.

Table 4

Results from LMM analysis of the effect of strongyle infection probability on log-transformed uCP levels, controlling for the effects of age, sex, rank, study year, and a quadratic effect of study week ($n = 400$ samples on 60 individuals). Individual ID was included as a random factor and all linear predictor z -transformed prior to analysis

	Estimate	SE	95% confidence intervals		z	P
Intercept	0.526	0.303	-0.068	1.119	1.736	0.083
Age	-0.238	0.110	-0.453	-0.023	-2.169	0.030
Sex	0.285	0.371	-0.443	1.012	0.767	0.443
Rank	0.082	0.185	-0.280	0.444	0.446	0.656
Study year	0.159	0.283	-0.394	0.713	0.564	0.572
Week	0.148	0.099	-0.047	0.343	1.490	0.136
Week ²	0.239	0.119	0.006	0.472	2.014	0.044
<i>Capillaria</i> probability	-0.167	0.100	-0.363	0.030	-1.664	0.096
<i>Strongyle</i> probability	-0.049	0.094	-0.234	0.135	-0.524	0.600

expected to induce immune responses and physiologically impact the host. Although parasitological studies and studies investigating which factors predict infection risk are common (Snaith et al. 2008; MacIntosh et al. 2012; Nunn et al. 2015; Rimbach et al. 2015), studies investigating impact of infections on individual health and behavior are still relatively rare to date (Ghai et al. 2015; Chapman et al. 2016; Friant et al. 2016a). Here, we show that strongyle nematode infections, despite not causing clinical symptoms in our study population, impact Barbary macaque physiology, activity, and social behavior.

Strongyle nematode infections were associated with significantly increased fGCM levels, a finding consistent with studies reporting higher GC levels correlating with GI parasite infections in non-human primates (Chapman et al. 2007; Muehlenbein and Watts 2010) and other taxa (Fleming 1997; Pedersen and Greives 2008). Similar to a recent experimental study showing reduced fGCM levels after antiparasitic treatment in mangabeys (Friant et al. 2016a), our results indicate that GI nematode infections might be the cause

of GC increases, although we cannot exclude the possibility that higher GC levels also increase GI nematode infection risk (Friant et al. 2016b) due to immunomodulatory effects of GCs (Cain and Cidlowski 2017).

Increases of GC levels can be interpreted in light of the immune challenge posed by strongyle nematode infections. Strongyle nematodes, including *Oesophagostomum*, cause inflammation and elicit parasite-specific immune responses (Pit et al. 2001; Andreasen et al. 2015; Andreasen et al. 2016). Typically, Th2 type, anti-inflammatory responses are linked to efficient immune defense against GI nematodes (Finkelman et al. 1997; Else and Finkelman 1998). GI parasites have been shown to modulate host immune system via manipulating cytokine profiles and shifting host immune responses more toward inflammation (Pit et al. 2001; Maizels and Yazdanbakhsh 2003; Hewitson et al. 2009). GCs, which are released in response to inflammatory processes, generally have anti-inflammatory effects and thus play a pivotal role in the regulation and termination of these inflammatory processes (Besedovsky et al.

Table 5

Results from the n/k binomial GLMM analysis of the effect of strongyle infection probability on activity, with n = active instantaneous records and k = inactive active instantaneous records for an individual each week ($n = 1528$ datapoints of 73 individuals), controlling for age, sex, rank, and the interaction between study group and study week (fitted as a quadratic term). Individual ID was included as a random factor and all linear predictors z -transformed prior to analysis

	Estimate	SE	95% confidence interval		z	P
Intercept	-1.013	0.085	-1.179	-0.846	-11.902	<0.001
Age	-0.183	0.042	-0.266	-0.100	-4.321	<0.001
Sex	-0.255	0.147	-0.543	0.032	-1.740	0.082
Rank	0.126	0.072	-0.015	0.267	1.751	0.080
Study year	-0.999	0.083	-1.162	-0.836	-12.012	<0.001
Week	0.288	0.009	0.270	0.306	30.854	<0.001
Week ²	-0.156	0.011	-0.177	-0.135	-14.437	<0.001
Week \times study year	-0.130	0.017	-0.163	-0.096	-7.641	<0.001
Week ² \times study year	0.411	0.019	0.375	0.448	22.000	<0.001
<i>Capillaria</i> probability	-0.021	0.010	-0.041	-0.001	-2.058	0.040
<i>Strongyle</i> probability	-0.015	0.009	-0.032	0.002	-1.728	0.084

Table 6

Overview numbers of directed approaches and departures. Counts of directed approaches and departures for each dyad ($n = 1298$ dyads), not controlling for observation time. Individuals are categorized into study years and experimental group. Interactions rates are not obviously different between the treatment and control group, however, as parasite clearance is relatively short for most individuals and the analysis is run comparing approach and depart frequencies within an individual with respect to parasite status, this pattern would be expected.

	Entire study period	pre	post1	post2	post3
Approach					
Range	0-64	0-64	0-35	0-24	0-52
Mean \pm SD	1.3 \pm 3.3	1.5 \pm 4.1	1.1 \pm 2.6	1.2 \pm 2.7	1.8 \pm 3.4
group C					
Range	0-64	0-64	0-35	0-24	0-52
Mean \pm SD	7.1 \pm 12.5	2.2 \pm 5.3	1.4 \pm 3.2	1.7 \pm 3.2	1.8 \pm 3.4
group H treatment					
Range	0-46	0-24	0-18	0-19	NA
Mean \pm SD	2.4 \pm 5.4	0.9 \pm 2.6	0.7 \pm 1.8	0.8 \pm 2.1	NA
group H control					
Range	0-51	0-24	0-30	0-24	NA
Mean \pm SD	2.5 \pm 5.5	0.8 \pm 2.2	0.8 \pm 2.2	0.8 \pm 2.2	NA
Depart					
Range	0-131	0-54	0-40	0-23	0-40
Mean \pm SD	4.5 \pm 9.1	1.5 \pm 3.9	1.0 \pm 2.4	1.2 \pm 2.6	1.8 \pm 3.3
group C					
Range	0-131	0-54	0-40	0-23	0-40
Mean \pm SD	7.2 \pm 11.8	2.3 \pm 5.1	1.4 \pm 2.9	1.7 \pm 3.1	1.8 \pm 3.3
group H treatment					
Range	0-36	0-23	0-15	0-16	NA
Mean \pm SD	2.4 \pm 4.7	0.9 \pm 2.2	0.8 \pm 1.7	0.7 \pm 1.8	NA
group H control					
Range	0-46	0-27	0-19	0-19	NA
Mean \pm SD	2.4 \pm 5.1	0.8 \pm 2.3	0.8 \pm 1.9	0.8 \pm 2.1	NA

1986; Kongsman et al. 2002). Thus, increased GC levels in response to GI nematode infections could reflect an advantageous response rather than a cost of infection. As indicated by the negative association between *Capillaria* and fGCMs, physiological effects are likely to be parasite-specific and can be impacted by parasites modulating immune responses to other taxa (Lello et al. 2004; Andreasen et al. 2015).

Another relevant mechanism could be tolerance to intestinal parasites as a host strategy to minimize costs of infections, for which GC levels can play a role (Medzhitov et al. 2012; Råberg 2014). Measuring tolerance versus immunity against parasites is difficult to achieve noninvasively, and we did not test for parasite tolerance

specifically in our study. Overall, low egg shedding intensities in combination with a prevalence of 100%, low rate of spontaneous parasite clearance, and high number of reinfections suggest that although individuals do not acquire complete protective immunity against *Oesophagostomum* (Bethony et al. 2006), they might trade off immune response versus parasite tolerance, accepting low-level infections (Medzhitov et al. 2012; Råberg 2014), especially because individuals are constantly exposed to infectious stages in the environment given their semi free-ranging housing conditions. Investigating whether GC levels mediate reinfection probability (Friant et al. 2016b) via impacting the immune system, or if moderate GC increases in infected individuals are linked to long-term

Table 7

Results of the negative binomial GLMM analysis of the effect of focal and partner strongyle infection probability on the number of directed dyadic approaches, controlling for the effects of age, sex, rank, study year, study phase, and dyadic CSI ($n = 8978$ dyadic measurements on 73 individuals). Focal individual ID and partner ID were included as random factors; log (focal hours) was included as offset term to control for sampling effort, all linear predictors were z -transformed prior to analysis

	Estimate	SE	95% confidence intervals		z	P
Intercept	-3.442	0.136	-3.709	-3.174	-25.218	<0.001
Age	-0.178	0.033	-0.242	-0.113	-5.389	<0.001
Sex	0.4089	0.129	0.157	0.662	3.178	0.001
Rank	-0.193	0.032	-0.256	-0.130	-6.001	<0.001
Study year	-0.835	0.167	-1.161	-0.508	-5.008	<0.001
Phase post 1	-0.156	0.074	-0.302	-0.010	-2.097	0.036
Phase post 2	0.218	0.052	0.117	0.319	4.229	<0.001
Phase post 3	0.321	0.082	0.161	0.481	3.934	<0.001
CSI	0.802	0.022	0.759	0.846	36.412	<0.001
Focal <i>Strongyle</i> probability	-0.017	0.030	-0.076	0.042	-0.569	0.569
Partner <i>Strongyle</i> probability	-0.058	0.030	-0.116	0.000	-1.955	0.051

Table 8

Results of the negative binomial GLMM analysis of the effect of focal and partner strongyle infection probability on the number of directed dyadic departures, controlling for the effects of age, sex, rank, study year, study phase, number of approaches received by the partner and dyadic CSI ($n = 8978$ dyadic measurements on 73 individuals). The final model included an interaction between individual approach numbers and strongyle infection status. Focal individual ID and partner ID were included as random factors, log (focal hours) was included as offset term to control for sampling effort, all linear predictors were z -transformed prior to analysis

	Estimate	SE	95% confidence intervals		z	P
Intercept	-3.586	0.081	-3.745	-3.427	-44.221	<0.001
Age	-0.134	0.030	-0.1933	-0.075	-4.449	<0.001
Sex	-0.157	0.063	-0.281	-0.033	-2.488	0.013
Rank	-0.010	0.029	-0.067	0.048	-0.330	0.742
Study year	-0.254	0.101	-0.452	-0.055	-2.506	0.012
Phase post 1	0.021	0.060	-0.097	0.140	0.356	0.722
Phase post 2	0.245	0.043	0.160	0.330	5.655	<0.001
Phase post 3	0.490	0.063	0.367	0.613	7.825	<0.001
CSI	0.176	0.015	0.145	0.206	11.428	<0.001
Approaches by partner	0.609	0.020	0.570	0.648	30.535	<0.001
Approaches to partner	0.441	0.018	0.405	0.477	23.938	<0.001
Focal <i>Strongyle</i> probability	-0.053	0.023	-0.098	-0.007	-2.276	0.023
Partner strongyle probability * approaches to partner	-0.009	0.024	-0.056	0.038	-0.378	0.705
Focal <i>Strongyle</i> probability	-0.036	0.015	-0.066	-0.006	-2.337	0.019

costs or benefits, will enhance our understanding of host–parasite interactions and the role of GCs. In contrast to strongyle infections, *Capillaria* infections occurred less frequently and were aggregated in young and aged individuals, suggesting resistance and responses aimed at parasite clearance, possibly explaining the negative relationship between infections and fGCM levels.

GC levels also play an important role in metabolism and increase in response to nutritional stress (Chapman et al. 2007), so increases in fGCM levels could also be mediated by GI nematodes via poorer nutritional status of infected individuals. This is likely not the case in our study population because physical condition, measured as uCP levels, was not significantly influenced by strongyle infections. Similarly, in a recent study in mangabeys, visually assessed body condition was more variable in individuals infected with the unicellular parasite *Balantidium coli* (Friant et al. 2016a) but not related to overall parasite richness. The lack of evidence for energetic costs of strongyle infections may come as a surprise, given the wealth of studies showing that higher parasite infection risks and/or intensities are linked with worse body condition and reduced nutritional intake across taxa (Coop and Holmes 1996; Díaz and Alonso 2003; Ezenwa 2004b; Irvine et al. 2006). We offer several nonmutually

exclusive explanations for the invariant energetic status of infected individuals in our study.

First, due to the ready availability of high-quality food in our provisioned population, energetic costs of infection and immune activation (Bonneaud et al. 2003; Derting and Compton 2003) may not lead to measurable energetic constraints. An alternative explanation is a potential impact of treatment by ivermectin, which has been shown to modulate glucose metabolism and reduce blood glucose in laboratory studies using mice (Laing et al. 2017), possibly masking the effect of parasite clearance on uCP levels. However, based on the short half-life of ivermectin (~36 h in humans) (Dourmishev et al. 2005), contrasted with prolonged periods of parasite clearance in some individuals, effects of ivermectin should be transient, whereas changes relating to infections should be more long term. The third and most likely explanation is mitigation of energetic costs of parasite infections by energy allocation: consistent with previous studies showing reduced activity and behavioral complexity in individuals excreting GI nematode eggs (MacIntosh et al. 2011; Ghai et al. 2015) or prior to antiparasitic treatment (Chapman et al. 2016; Friant et al. 2016a), individuals showed a trend to reduce activity when infected with

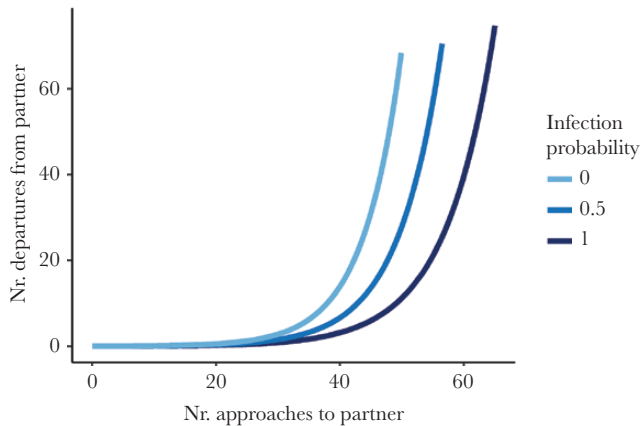


Figure 4

Effect of the interaction between approaches and strongyle infection probabilities on departures from an interaction partner. Estimates of departure numbers are plotted against the number of approaches from the individual to the respective partner. Each line represents the effect approach numbers have on departures at the respective strongyle infection probability. Although individuals depart more often from individuals they also approach more often, individuals depart the less from partners they approach often the higher their infection probability.

strongyle nematodes in our study. Decreased activity, like increased GC levels, is a characteristic of sickness behavior (Konsman et al. 2002; Dantzer 2004), induced by cytokine responses to infections, particularly to tissue damage and inflammatory processes common in strongyle infections (Bethony et al. 2006; Andreassen et al. 2015; Andreassen et al. 2016). Reduced activity, aimed at conserving body heat and energy (Hart 1988; Dantzer 2001), may suffice to maintain energy balance in the face of strongyle infections in provisioned macaques.

In contrast to strongyle infections, *Capillaria* infections were linked to lower uCP and activity, implying an effect on physical condition and sickness behavior in response to infections. As *Capillaria* was not reliably cleared by treatment, whether lower uCP and activity levels result from *Capillaria* infections or are markers of increased susceptibility to *Capillaria* cannot be distinguished in our study, yet our results add to the evidence suggesting a health impact of GI nematode infections even in the absence of overt symptoms in primates (MacIntosh et al. 2011; Ghai et al. 2015; Chapman et al. 2016).

In addition to investigating physiological consequences of infections, we assessed whether GI nematodes impact social behavior. We extend the current body of research by adding a dyadic perspective, whereas previous studies considered individual level interaction partner numbers or network integration measures and were thus unable to determine whether lower partner numbers in parasitized individuals are the result of sickness behavior or avoidance (Chapman et al. 2016; Friant et al. 2016a).

Despite evidence of sickness behavior (increased GCs and a tendency for decreased activity), individuals did not alter the frequency with which they approached interaction partners based on their own infection status. Rather, individuals seem to avoid spatial proximity to infected individuals, indicated by a reduction of approaches into proximity of infected partners compared with uninfected partners; directed approaches correlated strongly with the time spent in proximity with a partner. As this was a statistical trend only, this result needs to be interpreted with caution.

Considering that approach rates were generally lowest after treatment (when individuals showed the lowest parasite prevalence), probably based on seasonal effects, but infection probability was still linked with lower approaches directed to infected individuals, we are nonetheless confident this finding is based on actual adjustment of behavior in response to changing infection status of available partners.

Our results mirror those of experimental parasite clearance studies in vervet monkeys and mangabeys reporting an increase in number of partners in proximity following treatment (Chapman et al. 2016; Friant et al. 2016a). Given the propensity of frequent contact and central network positions to increase parasite infection risk (Fenner et al. 2011; MacIntosh et al. 2012; Rimbach et al. 2015; Wren et al. 2016), avoidance of infected conspecifics with subsequent lower exposure has been suggested as the main mechanism behind reduced social contacts in primates (Chapman et al. 2016; Friant et al. 2016a). As infected individuals do not always excrete eggs, whether this avoidance is directed toward excreting individuals or also those infected but not releasing eggs into the environment, remains an open question. Although a social component of GI nematode transmission has been proposed (Hernandez and Sukhdeo 1995), shared space use and subsequent increased contact with infectious stages via contaminated soil or food are considered as the major transmission route (Rimbach et al. 2015; Friant et al. 2016b), especially as eggs shed in feces are not immediately infectious but develop into infectious larvae in the environment (Bethony et al. 2006; Krief et al. 2010; Terio et al. 2016). Avoiding infected conspecifics might thus not be the best strategy to avoid GI nematode transmission. Infections with GI parasites have, however, frequently been linked to increased infection risk of various pathogens (reviewed in Cox 2001; Vaumourin et al. 2015), so we suggest that rather than specifically avoiding individuals infected with GI nematodes, individuals could avoid partners with a high overall susceptibility to infections to limit the risk of contracting various directly transmitted pathogens (Godfrey et al. 2009; Drewe 2010; VanderWaal et al. 2013).

The mechanisms by which infected conspecifics are detected remain largely unknown, yet olfaction probably plays an important role. From rodents to primates, individuals have been shown to detect infected conspecifics (Kavaliers and Colwell 1995; Poirotte et al. 2017), contaminated feces (Poirotte et al. 2017), or immune responses to infections (Olsson et al. 2014). Subtle behavioral changes due to sickness behavior, like reduced overall activity, may also serve as indicators of partner health and thus influence the propensity to interact. Further studies on how infections translate into signals of partner quality are needed for further clarification.

We predicted avoidance to be facilitated by departing from infected partners, yet individuals did not terminate proximity to infected partners more frequently. One interpretation of this result is that based on avoidance, infected individuals are approached rarely by most partners, leading to a ceiling effect on the departure level. Our finding that individuals leave partners the more the more these partners approached them suggests such a ceiling effect. Rather than leaving infected partners more often, individuals were less likely to leave others when they were themselves infected, an effect which was strengthened when individuals also frequently initiated proximity to the respective partner. Although this may be a result of generally reduced activity of infected individuals, we suggest that this result implies that infected individual compensates for being avoided by leaving proximity of partners less often. Beyond this effect, individuals seem to invest more in important

partnerships when infected, which mirrors results from laboratory studies reporting increased propensity to interact with valuable partners as a consequence of inflammatory cytokine signaling (Willette et al. 2007; Hennessy et al. 2014).

Reducing contact with infected conspecifics and limiting the number of interactions partners can reduce transmission risk and thus be beneficial (MacIntosh et al. 2012; Rimbach et al. 2015; Wren et al. 2016), but a lack of social interactions has implications for highly social animals like primates. Both strong social bonds and integration within the social network lead to short and long-term benefits and increased fitness (Silk et al. 2003; Schülke et al. 2010; Young et al. 2014; Brent 2015; Haunhorst et al. 2017; McFarland et al. 2017). Disrupting social bonds as a consequence of poorer health or to avoid interacting with infected partners could thus have detrimental effects on individual reproduction and survival. In mandrills, individuals tend to avoid grooming infected conspecifics but maintain grooming interactions with important partners (Poirotte et al. 2017). Infected individuals in our study appear to actively maintain social interactions despite the negative effects of GI nematode infections on their health. Both findings suggest that maintaining social relationships is important in the face of adversity and the potential health costs of contracting infectious diseases. Our study adds to the growing body of evidence that GI nematodes impact primate health and sociality and reinforces the idea of an evolutionary feedback loop between host behavior and parasite infection, with avoidance as a possible strategy to reduce infection risk.

SUPPLEMENTARY MATERIAL

Supplementary data are available at *Behavioral Ecology* online.

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Data accessibility: Analyses reported in this article can be reproduced using the data provided by (Müller-Klein et al. 2019).

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