Effects of Anthropogenic Habitat Disturbance and Giardia duodenalis Infection on a Sentinel Species' Gut Bacteria

Sahana Kuthyar[1], Martin Kowalewski[2], Dawn Roellig[3], Elizabeth Mallott[1], Yan Zeng[1], Thomas R. Gillespie[2], and Katherine Amato[1]

[1]Northwestern University
[2]Emory University
[3]CDC

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Abstract

Habitat disturbance, a common consequence of anthropogenic land use practices, creates human-animal interfaces where humans, wildlife, and domestic species can interact. These altered habitats can influence host-microbe dynamics, leading to potential downstream effects on host physiology and health. Here, we explored the effect of ecological overlap with humans and domestic species and infection with the protozoan parasite Giardia duodenalis on the bacteria of black and gold howler monkeys (Alouatta caraya), a key sentinel species, in northeastern Argentina. Fecal samples were screened for Giardia duodenalis infection using a nested PCR reaction, and the gut bacterial community was characterized using 16S rRNA gene amplicon sequencing. Habitat type was correlated with variation in A. caraya gut bacterial community composition but did not affect gut bacterial diversity. Giardia presence did not have a universal effect on A. caraya gut bacteria across habitats, perhaps due to the high infection prevalence across all habitats. However, some bacterial taxa, such as Actinobacteria, Bacteroidetes, Firmicutes, and Lachnospiraceae, were found to vary with Giardia infection. While A. caraya's behavioral plasticity and dietary flexibility allow them to exploit a range of habitat conditions, habitats are generally becoming more anthropogenically disturbed, and thus, less hospitable. Alterations in gut bacterial community dynamics are one possible indicator that A. caraya may be reaching its physiological limits for plasticity since changes in host-microbe relationships due to stressors from habitat disturbance may lead to negative repercussions for host health. These dynamics are likely relevant for understanding organism responses to environmental change in other mammals.

Introduction

Habitat disturbance, a common consequence of anthropogenic land use practices, can reduce survival and reproductive rates in some mammals, negatively affecting biodiversity (Arroyo-Rodríguez & Mandujano, 2006; Barelli et al., 2015; Luciana Ines Oklander, Kowalewski, & Corach, 2010). Traditionally, these effects have been linked to alterations in factors such as food availability and exposure to stressors, which can have direct physiological consequences (Chapman et al., 2006). Anthropogenically-sourced habitat disturbance is also frequently associated with greater ecological overlap between humans, wild animals, and domestic species, increasing the potential for disease transmission from humans and domestic species to vulnerable wildlife (Faust et al., 2018; Goldberg, Gillespie, Rwego, Estoff, & Chapman, 2008). Research suggests that these factors can influence mammalian physiology via interactions with the gut microbiome, more recently. For example, variation in diet across habitat are associated with differences in the gut microbiome of wild animals (Amato et al., 2013; Benítez-Malvido & Martínez-Ramos, 2003; Greene et al., 2019). Interactions among several host species within a shared environment may also facilitate the transmission of both commensal and

pathogenic microbes, thus modifying a host's microbial community structure (Moeller et al., 2013; Rwego, Isabirye-Basuta, Gillespie, & Goldberg, 2008). For example, humans, livestock, and non-human primates living in fragmented forests in Uganda shared similar strains of *Escherichia coli* , highlighting the potential for bacterial transmission in sympatric environments (Rwego et al., 2008). Given that the gut microbiome is known to affect host nutrition, metabolism, immune function, and behavior (Flint, Scott, Louis, & Duncan, 2012; Heijtz et al., 2011; Hooper & Macpherson, 2010), changes to its structure as a result of any of these pathways is likely to have substantial impact on host physiology and ultimately, reproductive success and survival.

Interactions between distinct groups of microbes in the gut may impact host health. Gut bacteria may affect host susceptibility to intestinal pathogen infection and influence the progress of pathogenic infection and clinical manifestation of disease (Berrilli, Di Cave, Cavallero, & D'Amelio, 2012; Costello, Stagaman, Dethlefsen, Bohannan, & Relman, 2012; Koch & Schmid-Hempel, 2011; Partida-Rodríguez et al., 2017). Alternatively, parasites may alter gut bacterial community composition, which could lead to alterations in host health (Barash, Maloney, Singer, & Dawson, 2017; Berrilli et al., 2012; Cantacessi et al., 2014; Peachey, Jenkins, & Cantacessi, 2017; Šlapeta, Dowd, Alanazi, Westman, & Brown, 2015). There is limited research on the interactions between gastrointestinal parasites and gut bacteria, and of those, many focus on mice, amphibians, or humans (Barash et al., 2017; Berrilli et al., 2012; Cantacessi et al., 2014; Cooper et al., 2013; Jani & Briggs, 2014; Kreisinger, Bastien, Hauffe, Marchesi, & Perkins, 2015; Lee et al., 2014; Shu et al., 2019). However, overall host-parasite-gut bacteria interactions seem to be system-specific. For example,*Cryptosporidium* spp. infection in captive Coquerel's sifaka has been associated with decreased gut microbial diversity as well as bacterial taxa linked to dysbiosis (McKenney, Greene, Drea, & Yoder, 2017). In contrast, domestic cats infected with the protozoan*Giardia duodenalis* have higher microbial species richness compared to uninfected individuals (Šlapeta et al., 2015).

Given that habitat disturbance often affects parasite prevalence and abundance patterns in wild mammals (Gillespie & Chapman, 2008), parasite-bacteria relationships may be a key factor for understanding mammalian health outcomes in anthropogenically-disturbed habitats (Cantacessi et al., 2014; Cooper et al., 2013; Kreisinger et al., 2015; Lee et al., 2014; Zaiss & Harris, 2016). Habitat disturbance may impact interactions between hosts and their associated microbial communities, which may then lead to downstream effects on host physiology and health, including nutritional deficits, higher prevalence of pathogens, and lower gut microbial diversity (Amato et al., 2013; Barelli et al., 2015; Estrada et al., 2017). As wildlife populations, including those of non-human primates, amphibians, and birds, are declining worldwide due to factors like habitat disturbance and zoonoses (Estrada et al., 2017; Rosenberg et al., 2019; Scheele et al., 2019), gaining more insight into the role of host-microbe relationships in mediating the outcomes of these health threats is integral to informing conservation practices. However, these interactions are understudied in wild animals.

Here we use the black and gold howler monkey, *Alouatta caraya* , as a model for exploring host-parasite-gut bacteria interactions in response to habitat disturbance. Primates are a relevant model in which to address these questions due to their large variation in habitat use, diet ecology, and inter-species interactions. As the most abundant primate species in northeastern Argentina, *A. caraya* is simultaneously a sentinel of ecosystem health and a model organism (Kowalewski & Gillespie, 2008, 2018; Kowalewski et al., 2011). For example, *A. caraya* experience high morbidity and mortality associated with yellow fever and thus serves as an early warning system prior to human outbreaks (Holzmann et al., 2010; Luciana Inés Oklander, Miño, Fernández, Caputo, & Corach, 2017).

Increasing anthropogenic activities in Argentina, such as deforestation and selective logging, are forcing *A. caraya* to live in ecological overlap with humans and domestic animals. *A. caraya* in these highly disturbed habitats interact with humans and domestic animals in multiple ways, including crossing terrestrially from forest patch to patch, sharing the same water sources as cattle, and engaging in altercations with domestic dogs (Kowalewski et al., 2011; Raño, Kowalewski, Cerezo, & Garber, 2016). These interactions may lead to an increased susceptibility to zoonotic diseases and higher sensitivity to gut dysbiosis via microbial transmission.

We examined the effect of ecological overlap with humans and domestic species – a proxy for anthropogenic

habitat disturbance – and infection by the protozoan parasite *Giardia duodenalis* on the gut bacteria of *A. caraya* . Disturbed habitats increase wildlife contact with humans and domestic animals and may result in a higher potential for cross-species, or zoonotic, microbial transmission (Gillespie & Chapman, 2008; Johnston et al., 2010). A previous study by Kowalewski and colleagues found a high prevalence of *Giardia duodenalis* in *A. caraya* populations across a gradient of disturbance (Kowalewski et al., 2011). As a result, we hypothesized that*A. caraya* in more disturbed habitats, with increased contact with humans and domestic animals, would have a higher infection prevalence of*G. duodenalis* . Additionally, research across animals, including amphibians, fish, and other species of *Alouatta* , indicates that habitat disturbance is associated with differences in gut bacterial community composition (Amato, 2016; Amato et al., 2013; Huang, Chang, Huang, Gao, & Liao, 2018; Sullam et al., 2012). Therefore, we hypothesized that anthropogenic disturbance would be associated with differences in the *A. caraya* gut bacterial community. Due to stressors that accompany habitat disturbance, such as changes in diet or decreased home range (Chapman et al., 2006), we predicted A.*caraya* in more disturbed habitats would have decreased gut bacterial diversity.

Finally, given reported interactions between parasites and gut bacteria (Barash et al., 2017; Berrilli et al., 2012; Cantacessi et al., 2014; Cooper et al., 2013; Jani & Briggs, 2014; Kreisinger et al., 2015; Lee et al., 2014; Shu et al., 2019), we hypothesized that *A. caraya*infected with *Giardia* would harbor a different gut bacterial community than uninfected individuals. In particular, based on studies of other primates (McKenna et al., 2008; McKenney et al., 2017), we predicted that *Giardia* infection in *A. caraya* would be correlated with decreased bacterial diversity and changes in bacterial community composition.

Materials and Methods

Study Site

This study was conducted in 2016 and 2017 across four sites in Corrientes and Chaco provinces in northeastern Argentina (**Table 1**): San Cayetano (27°34' S, 58°42' W), the Estacion Biologica de Corrientes and its surroundings (27°30' S, 58°41' W), Isla Brasilera (27°20' S, 58°40' W) and Cerrito (27°17' S, 58°37' W). Each *A. caraya* group, composed of three to 21 individuals, varied in their level of interaction with humans and domestic animals, as previously categorized by Kowalewski et al. (2011). Both San Cayetano and Cerrito are village habitats where *A. caraya* share environments with humans and dogs. Isla Brasilera is a remote habitat where *A. caraya* are mostly isolated in a flooded forest. The Estacion Biologica de Corrientes and its surrounding areas are characterized by a semi-deciduous forest in a matrix of grassland that is vulnerable to deforestation and these areas are classified as rural habitats, where*A. caraya* share environments with cattle (Kowalewski et al., 2011). All habitats are prone to flooding, and flash floods have been occurring more frequently in recent years, which could affect parasite prevalence and transmission. Notably, in April-May 2017, 600mm of rainfall across three days was recorded, leading to severe flooding.

Sample Collection

Fecal samples were collected from 63 individuals (remote = eight individuals, rural = 29 individuals, village = 26 individuals) non-invasively and opportunistically during the 2016 and 2017 winter seasons, following previous protocols (Gillespie, 2006). Sex, age class (infant, juvenile, sub-adult, or adult), social group, and habitat type of each individual were noted. Individuals were sampled for both*Giardia* presence and gut bacterial community characterization. For each sample, one gram of fecal matter was homogenized in sterile cryovials in RNAlater nucleic acid stabilizing buffer (Ambion, Life Technologies, Grand Island, NY) for *Giardia* analysis, and another gram was homogenized in sterile fecal vials with 95% ethanol for microbiome analysis. All samples were stored at 4°C until transport to the USA for processing, and all research procedures were approved by Emory University and Northwestern University (Northwestern IACUC Field Research 2019-001) and complied with applicable laws in Argentina. Import and export permits were obtained from the CDC and Argentina's Ministerio de Ambiente y Desarrollo Sostenible, respectively.

G. duodenalis analyses

All *Giardia* analyses were conducted at Emory University, Atlanta, Georgia. Due to the heterogeneity of

G. duodenalis , a multi-locus approach was utilized to target three genes: glutamate dehydrogenase (*gdh*), beta-giardin (*bg*), and triosephosphate isomerase (*tpi*). DNA was extracted from the RNAlater-preserved fecal samples using the FastDNA Spin Kit for Soil (MP Biomedicals LLC), and the multi-locus genes were amplified using a nested PCR protocol adapted from Roellig et al. (2015). Briefly, all PCR reactions were prepared in a final volume of 25µL containing 1x Taq PCR Master Mix (Qiagen), 400ng/µL BSA, 500nM of each primer, nuclease-free water and genomic DNA (2µL in first PCR reaction and 2µL of first reaction product in the second PCR reaction). **Table 2** lists the amplification conditions. Positive and negative controls were included in each reaction. Presence of *Giardia* infection was verified by running 5µL of PCR product on a 1% agarose gel.

Microbiome analyses

DNA was extracted from the ethanol-preserved fecal samples using the Qiagen Powersoil Kit at Northwestern University, Evanston, Illinois. The V4 region of the 16S ribosomal RNA gene was amplified using a modified version of the Earth Microbiome Project protocol (Mallott & Amato, 2018; Thompson et al., 2017) and the 515Fa/926R primer set (Walters et al., 2016). We barcoded and pooled amplicons in equimolar concentrations for sequencing on the Illumina MiSeq V2 platform at the DNA Services Facility at the University of Illinois at Chicago.

Paired-end sequences were joined and processed using QIIME2 v2019.7 (Bolyen et al., 2019). Quality filtering and the removal of chloroplast and mitochondria sequences resulted in a total of 856,786 reads with an average of 13,387 reads per samples. The DADA2 algorithm was used to cluster amplicon sequence variants (ASVs), and taxonomy was assigned by comparing ASVs to the GreenGenes 18_1 reference database. All samples were rarefied to 8,000 reads per sample based on alpha rarefaction curves **(Supplementary Figure 1a-c**). Alpha diversity (Faith's phylogenetic distance, Shannon diversity index, and number of observed OTUs) and beta diversity (unweighted and weighted UniFrac distances) were calculated in QIIME2.

Statistical Analyses

For *Giardia* analyses, infection prevalence per site was calculated as the proportion of individuals infected divided by the number of individuals sampled per site. Chi-square tests of independence were utilized to test if infection prevalence differed significantly across sites, and the p-value cutoff was at 0.05. All statistical analyses for microbiome data were performed with p-values cutoffs at both 0.001 and 0.05. Permutational analyses of variance (PERMANOVA) using the *adonis* function from the *vegan* package in R (R Core Team, 2014) were utilized to test for significant differences in bacterial community composition across habitats using both unweighted and weighted UniFrac distance matrices. Only eight samples from remote habitats were collected (due to flooding), so for subsequent analyses on alpha diversity and linear models, we filtered the dataset to include only samples from rural and village habitats and tested for differences due to habitat, group, year, and *Giardia* presence/absence, while controlling for individual. Additionally, the *nlme* package in R was used to run linear mixed effects (LME) models to examine the effects of habitat and *Giardia* presence, separately, on alpha diversity indices. LME models were also used to test for effects of habitat and*Giardia* presence on the relative abundance of bacterial phyla, family, and genera, while controlling for individual identity. Finally, linear discriminant analysis effect size (LEfSe) through Galaxy was utilized to understand which bacterial taxa discriminated each habitat type (Afgan et al., 2018; Segata et al., 2011).

Results

Giardia infection

Total *Giardia* prevalence was 82.6% (52/63 individuals) across all habitats. Almost all individuals were infected with *G. duodenalis* in rural (87.9% (26/29 individuals)) and village (88.5% (23/26 individuals)) habitats, yet only 37.5% (3/8) of *A. caraya*were found to be infected with *Giardia* in remote habitats. There was no significant difference in infection prevalence between *A. caraya* in rural and village habitats. However, the differences in infection were significant between *A. caraya* in remote and rural habitats (x^2 =10.06, df = 1, p-value<0.05) as well as those between individuals in remote and village habitats (x^2=8.83, df = 1,

p-value<0.05).

Habitat disturbance and gut bacteria

Bacterial community composition varied across habitats (**Figure 1** ; unweighted UniFrac: PERMANOVA $F_{1,2}$ = 3.22, R^2 = 0.0963, p-value<0.001, weighted UniFrac: PERMANOVA $F_{1,2}$ = 10.4537, R^2= 0.238, p-value<0.001) and social groups (unweighted UniFrac: PERMANOVA $F_{1,15}$ = 1.37, R^2 = 0.307, p-value <0.001, weighted UniFrac: PERMANOVA $F_{1,15}$ = 1.80, R^2=0.307, p-value<0.001). There was no difference in any of the richness or diversity indices with respect to habitat type or social group (all p>0.05). However, we found several bacterial taxa driving overall differences in gut bacterial community structure across habitats. Using LME models, we observed *Bacteroidetes* was significantly higher in rural habitats relative to village habitats ($F_{1,46}$ =9.386, p-value<0.05), whereas*Firmicutes* was significantly higher in village habitats relative to rural habitats ($F_{1,46}$ = 4.50, p-value<0.05). Both *Prevotellaceae*($F_{1,46}$ = 14.26, p-value<0.001) and*Prevotella* ($F_{1,46}$ = 14.39, p-value<0.001) were more abundant in rural habitats than in village habitats.

We also ran a linear discriminant analysis model (LEfSe) to identify the bacterial taxa that were differentially abundant at the family level (**Figure 2**). We found *A. caraya* in rural habitats were enriched with *Erysipelotrichaceae* and *Lachnospiraceae,*whereas *A. caraya* in village habitats were enriched with*Ruminococcaceae* .

Giardia infection and gut bacteria

Despite the high prevalence of *Giardia* , infection was not associated with variation in overall gut bacterial community composition within or across habitats (all p-value>0.05). Further, Shannon diversity and Faith's phylogenetic distance did not correlate with *Giardia* infection. However, bacterial richness (observed OTUs) varied significantly with *Giardia* infection ($F_{1,46}$ = 8.42, p-value<0.05). On average, there were less observed OTUs in *Giardia* -infected individuals when compared with uninfected individuals. Additionally, certain taxa were modestly yet significantly associated with *Giardia* presence, including *Actinobacteria* ($F_{1,46}$ = 4.35, p-value<0.05), *Bacteroidetes* ($F_{1,46}$ = 4.86, p-value<0.05), *Firmicutes* ($F_{1,46}$= 3.99, p-value<0.05), and *Lachnospiraceae*($F_{1,46}$ = 6.52, p-value<0.05).

Discussion

This study examined the effects of habitat disturbance and *G. duodenalis* infection on the *A. caraya* gut microbiota in northeastern Argentina. As hypothesized, we found that habitat type was significantly associated with differences in both *Giardia*infection prevalence and gut bacterial community composition. There was also a significant interaction of *Giardia* infection with the relative abundance of specific bacteria taxa and within sample bacterial diversity. These results suggest that examinations of gut bacteria-parasite interactions are important to include in studies of host physiological responses to environmental change.

Habitat disturbance and G. duodenalis

Habitat disturbance leads to increased contact between wildlife, livestock, and humans, which increases the risk for zoonotic disease transmission. For example, deforestation in Australia has been associated with the emergence of bat-borne viruses, like Hendra viruses (Field, 2009). In our study, habitat differences in ecological overlap were associated with differences in *G. duodenalis* prevalence.*G. duodenalis* prevalence in *A. caraya* was higher in rural and village habitats compared to remote habitats. This pattern may be a result of increased contact with humans and livestock. *A. caraya*share environments with cattle in rural habitats and with humans and dogs in village habitats, whereas they are mostly isolated in remote habitats. This variation in interaction may put *A. caraya* in rural and village habitats at higher risk for cross-species transmission of *Giardia* spp. and other parasites. Further, *A. caraya* in rural habitats display higher rates of terrestriality due to habitat fragmentation, as individuals cross on the ground between patches of forest, leading to increased interaction with both animals and infective parasite cysts and thus, a higher frequency of infection.

In these rural and village habitats, we also detected a difference in*G. duodenalis* prevalence across years. Due

to a massive flood in April 2017, we were not able to collect samples from remote habitats that year, limiting our sample size for comparison. *Giardia*infection was previously screened for in 2011 and 2016 in this *A. caraya* population, however, and prevalence in both rural and village habitats was significantly lower during these years compared to our data from 2017 (**Figure 3**) (Kowalewski et al., 2011; Kuthyar, unpublished data). Although previous studies have found gastrointestinal parasitic infection in howler monkeys living in disturbed habitats (Eckert et al., 2006; Trejo-Macías & Estrada, 2012; Trejo-Macías, Estrada, & Mosqueda Cabrera, 2007; Vitazkova & Wade, 2006), the high infection rates found in rural and village habitats in 2017 most likely resulted from water contamination from the 2017 flooding event, as all sampling sites were inundated. High humidity favors the survival of the infectious stages of *Giardia* cysts (Martíez-Mota, Kowalewski, & Gillespie, 2015), and parasite prevalence has been associated with high levels of precipitation in *A. caraya* habitats (Kowalewski & Gillespie, 2008). *Giardia* cysts are also more infectious for a longer period of time in water than in soil and feces (Olson, Goh, Phillips, Guselle, & McAllister, 2010), so flooding may have spread infective *Giardia* cysts across rural and village habitats. As climate change is increasingly impacting the landscape and forest fragmentation, cycles of flooding have changed from every 15 years to every two or three years. The increased *Giardia* prevalence in 2017 may reflect this change in the flooding cycle and may predict future alterations in *Giardia* dynamics in the ecosystem.

Habitat disturbance and gut bacteria

Habitat type was also associated with variation in the *A. caraya*gut bacterial community, where different habitats were associated with different gut bacterial community compositions. Multiple factors may contribute to these patterns, including diet, intra- and inter-species contact, and the physical environment (Mccord et al., 2014).

Several bacterial taxa varied between individuals living in rural and village habitats, including *Prevotella* , *Lachnospiraceae* , and *Ruminococcaceae* – a pattern that may be associated with diet. Previous studies have shown that differences in diet composition, and thus nutrient availability, across habitats influence the types of gut bacteria residing in animal hosts (Amato et al., 2013; Barelli et al., 2015; Trosvik, Rueness, De Muinck, Moges, & Mekonnen, 2018). For example, cloacal bacterial composition in juvenile green turtles differs between habitats as a result of shifts in diet (Price et al., 2017). In some studies, the same bacterial taxa in which we detected variation responded to diet. High abundances of *Prevotella* have been found in vervets and macaques eating a Western diet (Amato et al., 2015; Ma et al., 2014), red colobus monkeys in protected forest in Tanzania (Barelli et al., 2015), and black howler monkeys consuming a leafy diet (Amato et al., 2019, 2014). *Ruminococcaceae* relative abundances are reported to be higher in red colobus monkeys in disturbed habitats (Barelli et al., 2015), in black howler monkeys during seasons of low energy intake (Amato et al., 2014), and in frogs with low dietary diversity in farmland habitats (Chang, Huang, Lin, Huang, & Liao, 2016). Similar dynamics between these bacterial taxa and diet may be operating in our system. In *A. caraya* at our site, differences in food availability have previously been documented between the remote, rural, and village habitats (M. Kowalewski & Zunino, 2004; Zunino, González, Kowalewski, & Bravo, 2001). There are differences in overall seasonal patterns of food availability as well as the availability of new and mature leaves across habitats (Kowalewski & Zunino, 2004). Additionally, lower food abundances have been recorded in rural and village habitats compared to remote habitats (Zunino et al., 2001), and individuals in village habitats sometimes receive fruit and bread from residents (M. Kowalewski, personal observation. All of these differences have the potential to affect competitive interactions between bacteria in the gut, thereby altering community composition overall. However, since we did not collect diet data in this study, it is unclear if previously documented differences in food availability correlate to differences in actual food consumption across sites during our study. Future studies should incorporate *A. caraya* diet data to see which dietary factors (i.e. leaf abundance, human food scraps, or fruit abundance) drive these changes in bacterial taxa across habitats.

Interestingly, there was no difference in bacterial richness or diversity across habitat types in *A. caraya* . This finding is in contrast to other studies in a range of animals. For example across various species of primates, habitat fragmentation has a modest by significant effect on gut bacterial diversity (Amato et

al., 2013; Barelli et al., 2015; Mccord et al., 2014). Additionally, house sparrows in urban environments were characterized by decreased microbial diversity and fewer metabolic functions (Teyssier et al., 2018). These patterns are often attributed to variation in diet since diet diversity is linked to gut microbiome diversity (Heiman & Greenway, 2016), and habitat disturbance often alters diet diversity as a function of food availability. Indeed, reduced dietary diversity is associated with reduced bacterial diversity in both red colobus monkeys and howler monkeys in fragmented and/or disturbed habitats (Amato et al., 2013; Barelli et al., 2015). However, habitat disturbance and fragmentation may not impact the interaction between diet and gut bacterial structure in the same way across all species. For example, vampire bats exhibit less diverse diets in fragmented habitats but show no changes in gut bacterial diversity between habitats (Ingala, Becker, Bak Holm, Kristiansen, & Simmons, 2019). Furthermore, in the disturbed rural and village habitats of this study, *A. caraya* diet composition may be more diverse compared to diets observed in remote habitats (M. Kowalewski, personal observation) as a result of edge effects and secondary forest succession after selective deforestation (Kowalewski & Zunino, 1999). Future research should include data on actual food consumption to further explore the interaction between diet diversity and gut microbial diversity.

Beyond diet, patterns of microbial transmission may also contribute to the cross-habitat differences in bacterial community composition identified in this study. Evidence of these dynamics have been reported previously. For example, gorillas that lived in ecological overlap with humans and livestock in Rwanda harbored similar strains of *E. coli* to those of humans and livestock compared to gorillas that did not overlap (Rwego et al., 2008). Further, a study in Uganda found that *Cryptosporidium* spp. isolates looked genetically identical regardless if it came from a human, non-human primate, or livestock source (Salyer, Gillespie, Rwego, Chapman, & Goldberg, 2012). Here, as high ecological overlap exists among *A. caraya* , humans, and domestic animals in the anthropogenically-disturbed habitats, *A. caraya* in fragmented rural and village habitats may be more exposed to cross-species bacterial transmission, thus altering their bacterial community structure, than at remote habitats, where they are mostly isolated from humans and livestock.

Finally, the physical environment and the resulting exposure to environmental bacterial pools may shape a host's gut bacteria across habitats. In a previous study, soil was found to best predict the gut bacteria of baboons in Kenya (Grieneisen et al., 2019). Since baboons are terrestrial and *A. caraya* are arboreal, soil itself may not have as strong an influence on the *A. caraya* gut bacterial community in our study. However, contact with other substrates in arboreal habitats could have a similar effect. Furthermore, some of the *A. caraya* groups sampled in this study are commonly reported to travel on the ground between patches of fragmented forest at rural sites (M. Kowalewski, personal observation). This behavior is likely to increase their contact with terrestrial substrates (e.g. soil and animal feces) and may lead to patterns of environmental bacterial acquisition similar to those observed in the baboons. Moving forward, we hope to measure soil samples to test this mechanism.

Gut bacteria and G. duodenalis

As hypothesized, *G. duodenalis* infection was associated with differences in gut bacterial community membership. Past studies in frogs found that parasitic infection led to altered skin bacterial communities and even increased the pathogen load in some cases (Jani & Briggs, 2014; Shu et al., 2019). In *A. caraya,* we found that some bacterial taxa, such as *Actinobacteria* , *Bacteroidetes* ,*Firmicutes* , and *Lachnospiraceae* , varied with *Giardia* infection. These taxa are all associated with providing crucial metabolic services to the host (Biddle, Stewart, Blanchard, & Leschine, 2013; Binda et al., 2018; Johnson, Heaver, Walters, & Ley, 2017), and so, changes in their abundance could have impacts on howler health beyond susceptibility to infection and symptoms of disease. The modified bacterial community structure, along with living in a disturbed habitat and harboring a parasite, could further alter the nutritional, and thus overall health, status of *A. caraya* in these contexts.

Bacterial richness was also significantly associated with infection, where infected individuals had fewer OTUs on average compared to uninfected individuals. It is possible these reductions in diversity could enhance the potential for colonization by potentially pathogenic microbes, such as *Enterococcus* sp. (Iebba et al., 2016). However, more research is necessary. Previously, parasite infection was associated with decreased

microbial diversity in birds in the Galapagos, where initial infection led to changes in host behavior, leading to further susceptibility to future infection (Knutie, 2018). However, infection with chytrid fungus in frogs was associated with increased bacterial diversity (Becker, Longo, Haddad, & Zamudio, 2017). In past studies, *Giardia* has been associated with bacterial overgrowth as well as changes in the relative abundance of certain taxa, including increases in *Proteobacteria* and decreases in *Firmicutes*and *Bacteroidetes* (Barash et al., 2017; Halliez & Buret, 2013; Müller & Von Allmen, 2005). Additionally, dogs infected with*Giardia* had significant differences in their bacterial community structure compared to un-infected dogs (Šlapeta et al., 2015). The implications of these changes to host health both in the present study and across the literature remain to be seen.

It is also important to note *Giardia* infection did not seem to have a universal effect on the *A. caraya* gut bacteria across habitats. For example, individuals in rural habitats with high infection prevalence were significantly enriched with *Lachnospiraceae* , a pattern not observed in village sites. Since *A. caraya* exhibited distinct gut bacterial communities across habitats, the composition of their microbiomes in each type of habitat could increase or decrease their susceptibility to *G. duodenalis* . Further, due to these differences in gut bacterial communities, host physiological responses to parasitic infections, such as *Giardia* and other gastrointestinal parasites, could vary with habitat type. Presence of infection may also impact host response to habitat disturbance as individuals infected with *Giardia* may or may not survive better in a given habitat depending on the parasite's effects on the gut bacterial community.

A number of factors constrained this study. Due to the flooding event in 2017, we were not able to collect fecal samples from *A. caraya*individuals living in the remote habitat, which limited the comparison of *Giardia* infection across habitats. Additionally, we did not record specific measures of host health, such as stress levels or immune functioning, which may have demonstrated if changes in gut bacterial communities due to habitat disturbance and/or *Giardia* infection impacted *A. caraya* health. A more robust sample size both within habitats as well as across years would provide a more informed understanding of how habitat disturbance and *Giardia* infection affect the *A. caraya* gut microbiome. Further, since *G. duodenalis* is a multi-species complex with eight distinct genotypes and degrees of virulence (Ryan & Cacciò, 2013), future research should explore if different *Giardia* genotypes have different interactions with the gut microbiome, and thus consequences for host health.

Overall, this study indicates that anthropogenic habitat disturbance influences multiple groups of mammalian gut microbes and that these microbes also affect each other. Understanding the influences these interactions have on host health outcomes in disturbed habitats will be important for conservation efforts moving forward. Habitat disturbance may not only affect host health through shifts in food availability and quality, but also indirectly through changes to host-associated bacterial communities and susceptibility to parasite infection. Further, integration of the gut microbiome into a disease ecology framework will be crucial in understanding the intrinsic and extrinsic factors that impact host survival and reproduction.

As a sentinel species of this semi-deciduous ecosystem, *A. caraya*plays a crucial role in advising wildlife health surveillance of increasingly fragmented and disturbed habitats. Their behavioral plasticity and dietary flexibility allow them to exploit a large range of habitat conditions, resulting in resilience in fragmented and disturbed habitats (Kowalewski et al., 2011; Miner, Sultan, Morgan, Padilla, & Relyea, 2005). As habitats are becoming harsher due to anthropogenic pressures, however, previously resilient wild animals may be reaching their limits of plasticity. In addition to more obvious factors, the understudied interactions between habitat disturbance, pathogen transmission, and a host's gut bacteria could negatively influence host health, reproductive output, and even survival. As such, monitoring both the host's gut bacterial community and pathogen load could be an important non-invasive method to improve understanding of the effects of anthropogenic disturbance on wild mammal health and inform conservation strategies.

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Data Accessibility

Scripts for QIIME2 and R can be found at https://github.com/Kramato-lab/kuthyar_giardia. The datasets used for analyses can be accessed via Dryad at https://doi.org/10.5061/dryad.wm37pvmj5. All sequences have been uploaded to SRA and will be publicly accessible with publication (PRJNA607274).

Author Contributions

S.K. analyzed the *Giardia* samples, with help from D.M.R and T.R.G, and was funded through National Geographic and Emory University. S.K., Y.Z., and E.K.M analyzed the microbiome samples and were funded through Northwestern University. S.K. analyzed the data with supervision from E.K.M, K.R.A., and T.R.G. M.M.K, K.R.A, and T.R.G. helped supervise the project. This project is also funded by a Goldberg Research Grant from the Nacey Maggioncalda Foundation. K.R.A is supported as a CIFAR Azrieli Global Scholar. All authors contributed to the final manuscript.

Tables and Figures

Table 1, Sampling sites

Site	Habitat characterization	Human-associated species that
San Cayetano	Village	Humans and dogs
Estacion Biologica de Corrientes and surrounding areas	Rural	Cows
Isla Brasilera	Remote	None
Cerrito	Village	Humans and dogs

Table 2, Thermocycler conditions for nested PCR amplification of *G. duodenalis*

Gene	Fragment Amplified	PCR conditions
gdh	599bp	Initial denaturation at 94°C for 3 minutes; 35 cycles of denaturation at 94°C for 45 secon
tpi	530bp	Initial denaturation at 95°C for 2 minutes; 35 cycles of denaturation at 95°C for 1 minute

Gene	Fragment Amplified	PCR conditions
bg	511bp	Initial denaturation at 94°C for 3 minutes; 35 cycles of denaturation at 94°C for 20 secon

Figure 1 , Beta diversity across habitats based on the weighted Unifrac distance matrix.

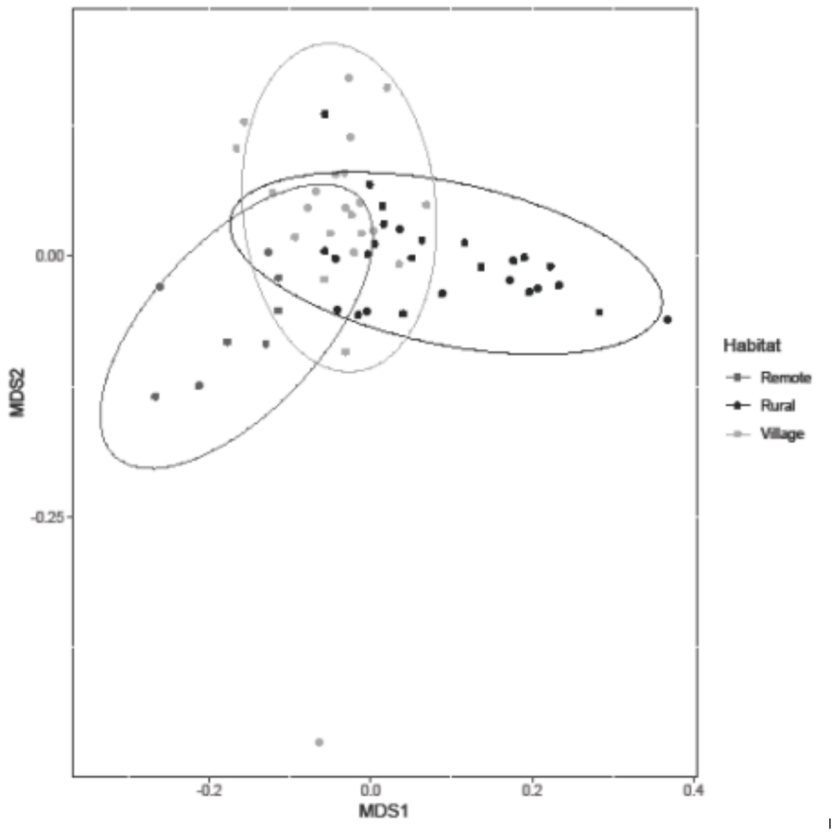

Figure 2 , Average relative abundance of bacterial taxa differentially expressed between rural and village habitats.

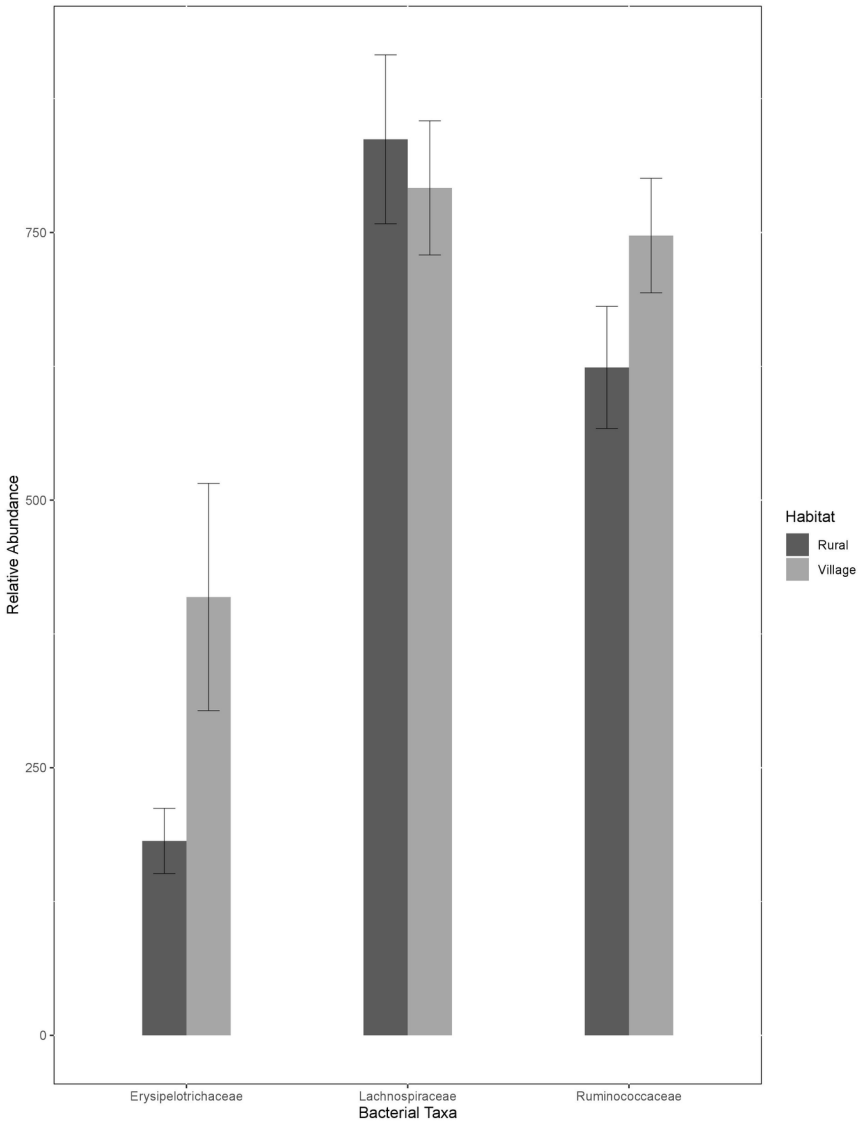

Figure 3 , *Giardia duodenalis* prevalence in *A. caraya* across years and habitats.