A Comparative Analysis of Gut Microbiota on the Human-Macaque Interface in Northeast Thailand

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Abstract

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Traditional zoonotic disease research efforts centered on detection of high profile pathogens may miss opportunities to understand broader microbial transmission dynamics between humans, animals, and the environment. The Global Assessment of Zoonotic and Environmental Risks (GAZER) platform seeks to address this knowledge gap by examining overlaps of bacterial microbiome communities between humans, animals, and environments in settings where interaction with animals is high and potential for human health impacts of this contact are greater. This thesis presents data from Maha Sarakham, Thailand, where a growing population of long-tailed macaques (*Macaca fascicularis*) in the Kosumpee Forest Park interface with residents of the adjacent village. In particular, community members working in or near the park experience a high level of direct and indirect contact with macaques through feeding as well as aerosols of macaque feces during cleaning. Workers were surveyed to characterize tasks that

contribute to exposure and other dietary or lifestyle factors that influence gut microbiome composition. We employed comparative microbiome analysis based on the V4 region of the 16S rRNA gene from DNA extracts of stool samples to assess the degree of similarity between gut bacterial communities and potential for pathogen transmission between macaques and workers. Fecal samples were collected from humans (exposed, n=12; control, n=6) and macaques (exposed, n=8; control, n=4) using the OMNIgene.GUT kit and sequenced on the Illumina HiSeq platform. SourceTrackers was the primary tool to assess degree of microbial sharing between humans and macaques and revealed no significant difference in microbial sharing with macaques between exposed and control humans. Variance detected in PCoA visualizations of the unweighted UniFrac distance were tested using adonis and betadisper to investigate the potential role of the Anna Karenina principle (AKP). Exposed macaque samples exhibited significantly greater dispersion than controls ($p<0.01$). Human samples had homogenous dispersion but different spatial medians between groups ($p<0.03$), implying a shift in microbial composition. Alterations in gut microbiota of exposed macaques highlights the potential for increased susceptibility to other diseases. Task observations and surveys assessing knowledge, attitudes, and practices among workers revealed opportunities to employ of protective measures or training to reduce exposure to occupational hazards. This information can also be used to mitigate negative aspects of contact between humans and macaques in order to optimize the health of both populations.

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1. BACKGROUND

Habitat fragmentation and human encroachment results in a patchwork of isolated non-human primate populations across Thailand. Supplemental feeding for religious reasons or tourism contributes to a growing macaque population unconstrained by natural food resources. While some locals view monkeys as destructive pests and see no benefit to their presence, others feel that, while monkeys' actions can be frustrating, they ascribe to the Buddhist belief that the monkey is sacred and therefore they seek to coexist with and protect them [1]. Macaques may also be an important source of income or economic stability due to their appeal to tourists. Amidst these changes, the growing level of human-macaque conflict has led researchers to call for improved management plans and conservation strategies [2, 3]. Ensuring local populations are educated regarding potential health risks associated with macaque contact has been cited as a possible disease prevention strategy that will likely become more relevant as monkey-based tourism grows [4].

In Kosumpee Forest Park of northeastern Thailand, park workers care for a growing population of long-tailed macaques (*Macaca fascicularis*) by supplementing their diet with bananas every day or every other day. Additionally, some workers collect monkey carcasses (typically from vehicle accidents) and remove them from the park. Others, including janitors at the local school, are responsible for sweeping debris from under monkey roosting sites, which aerosolizes fecal matter. These tasks, along with an overlapping environment with macaques, put these workers at an increased risk of zoonotic disease transmission relative to other members of the Kosum Phisai community. Community members can and do visit the forest park, they tend to drop food offerings in a manner that puts them in minimal contact with macaques [1].

Due to their genetic similarity, humans and macaques are susceptible to many of the same infectious diseases. In recognized high risk settings, like laboratories, workers receive training on the numerous zoonoses that can be transmitted between humans and macaques, including herpes B virus, poxvirus, and simian foamy virus [5]. Parasitic infections from soil-transmitted helminths such as *Strongyloides fuelleborni* and *S. stercoralis* have also been documented in humans and non-human primates in this region and are typically maintained in macaques and humans, respectively [6]. Some of these zoonoses are classified as emerging or re-emerging due to the increased degree of human-wildlife contact, which raises the chances of infectious diseases spilling over from wild primate populations and causing outbreaks in the community. Workers in and around Kosumpee Forest Park represent a critical control point for spread of zoonoses to the broader community. However, unlike in the laboratory setting, Kosum Phisai workers' knowledge, attitudes, and practices surrounding these occupational health risks are poorly characterized [7].

Comparative analyses of gut microbiota between workers and macaques may shed light on the degree of transmission of microbes that is occurring between human and non human primates in close contact settings, and whether there may be health consequences related to such transmission. Recent studies have demonstrated that the community composition of microbiota is influenced by our environment and the animals we interact with, and the degree of sharing can be quantified using Bayesian approaches like SourceTracker [8-10].

Many of the tasks these workers perform involve aerosolizing macaque fecal material and it is possible that macaque fecal microbes are introduced to worker gastrointestinal tracts through this ingestion route. In addition to the richness of information contained in gut microbiome samples, other relevant advantages of analyzing the gut microbiome include the fact that stool microbiome samples are relatively easy to collect in a standardized fashion, better characterized

in literature, more temporally stable, and yield higher read counts than other microbiome sites including the skin microbiome [11]. The hypothesis of this study is that Workers who experience overt exposure to macaques and the microbes they carry will exhibit gut microbiome profiles that contain a greater number of microbes that are also found in macaques compared to control individuals who lack exposure to macaques (occupationally or recreationally).

Based on task observations, it may be possible to propose intervention strategies to reduce exposure to macaque biological material. Such strategies could include the use of personal protective equipment, training, or other controls with the intent of mitigating the risk of disease spillover from the primates into human populations as well as protecting macaque populations from pathogens the workers may transmit through reverse zoonotic transmission.

2. METHODS

Study Design: This study has a cross-sectional design, with stool sample collection and survey administration to controls and exposed populations of humans and macaques over at a single point in time. The general study design, however, could form the basis of a baseline assessment that could be extended in the future to continued, longitudinal surveillance of these high risk worker populations.

Study setting: The study site for exposed participants was a village adjacent to Kosumpee Forest Park (KFP), Kosum Phisai District, Maha Sarakham Province in northeastern Thailand (16°15'19"N 103°04'06"E). The forest park adjacent to the village covers an area of approximately 0.2 km^2, bordered on the east by the Chi River. The forest park contains over 700 long-tailed macaques, divided into 5 social groups with largely overlapping ranges, which served as the macaque exposed population [2]. Control sites were Mahasarakham University for

humans, approximately 24 km E of KFP and a forest in Phon Ngam (16°21'01"N 102°56'54"E) for macaques, approximately 16 km NW of KFP, where there is little human-macaque interaction.

Study subject inclusion criteria and recruitment: Eligible workers (n=12) were defined as members of the community who contact monkeys or monkey bodily fluids (blood, feces, urine) as a component of their paid work at least once per week.. Workers were excluded if they had not worked at that site for a minimum of 3 months. Human controls (n=6) were recruited from a convenience sampling of students at the nearby Mahasarakham University and were determined eligible if they were over 18 years of age and had no contact with macaques. Recruited participants were explained the study objectives and offered 100 Thai baht as compensation for their time.

Exposed macaques (n=8) were sampled by RK at Kosumpee Forest Park, with an effort to collect samples from monkeys belonging to each of the social groups (Red Dot, Hare Lip, Stump Tail, Droop Lip) and age/sex distribution representative of the overall population. These monkeys are individually identifiable by facial features or other unique characteristics by RK. Control macaques (n=4) were sampled from a nearby forest in Phon Ngam in the same manner as exposed macaques, and age/sex were recorded.

Ethics Statement: The research in this study was approved through the University of Washington Institutional Review Board (IRB) for human subjects research and Institutional Animal Care and Use Committee (IACUC) for animal research (#51546 and #3143-04, respectively). The study also received approval through Mahasarakham University for human and animal subjects research (protocol numbers 037/2016 and 0009/2016, respectively). Human participants were informed that their participation was voluntary, that they could withdraw at any

time, and that questionnaire responses, individual microbiome results, and task observation videos would be kept confidential or de-identified. Macaque samples were obtained from fresh defecations, therefore no direct macaque contact occurred as part of this study.

Data collection: Macaque workers were surveyed by PK regarding practices that may increase their opportunities for exposure to pathogens, training (e.g. monkey behavior, PPE use, wound care) and their knowledge of the principle that macaques and humans can share diseases. The GAZER (Global Assessment of Zoonotic Exposure Risks) survey template was translated into Issan Thai by PK, was used to assess current training, practices, and knowledge regarding disease transmission between workers and macaques. We piloted the survey used in this study for eight workers in October 2016 and revised the survey to address limitations that emerged during administration and analysis. Additions included a dietary questionnaire based on a modified food frequency questionnaire (FFQ), which is commonly used to characterize diet in studies of the gut microbiome.

Task observations of workers were collected by study team members PK and RK using a GoPro video recorder in order to provide an assessment of work activities and supplement characterization of exposure opportunities obtained in the survey. The two hour video recordings were reviewed by two individuals to maintain consistency in describing and quantifying observed tasks according to the Table S1 in Supplemental Materials.

Interviews, task observation, and sample collection was conducted from Sept 24 – Oct 7, 2017. Survey data and sample metadata were collected and managed using REDCap electronic data capture tools hosted at University of Washington [3].

Sample Collection and Processing: The fecal samples were placed immediately after sampling in vials from the OMNIgene.GUT kits (DNA Genotek, Ontario, Canada) to stabilize

and preserve community composition in the absence of a cold chain. Workers were provided with sterile collection kits and instructions for proper specimen collection; macaque samples were similarly collected using sterile tools from the center of fresh excrement. These samples were transported to a lab at Khon Kaen University and stored at ambient temperature for no longer than 30 days from the date of collection prior to DNA extraction. QIAamp PowerFecal DNA Isolation kit (Qiagen, Hilden, Germany) was used to extract genomic DNA, following manufacturer protocols.

DNA concentration was determined using a NanoDrop2000 spectrophotometer (NanoDrop Technologies Inc., DE, USA) and the integrity of DNA was evaluated by running 5 ul of sample on a 0.8% agarose gel under 100 V for 30 min. Extracted DNA samples were shipped overnight on blue ice to Genewiz Laboratories in Suzhou, China. DNA quality was verified by Genewiz using NanoDrop, Qubit, and agarose electrophoresis. The V4 region of the bacterial 16S genes were amplified using the 515F-806R primers, based on the Earth Microbiome Project protocol [4]. Amplicons were sequenced on an Illumina HiSeq platform by Genewiz Laboratories.

Data Analysis: DNA sequences or reads in the form of FASTQ files were analyzed with QIIME2 version 2017.12.0 pipeline [5]. DADA2 version 2017.12.1 [6] was used for sequence quality control and feature table construction. Forward reads were truncated to 280 bp and reverse reads to 260 bp. Samples were rarefied to the lowest sample depth of 12,466 reads per sample and diversity metrics calculated accordingly [9]. Sequences were assigned taxonomy using the Greengenes 13_8 reference database [10]. We also assigned taxonomy using SILVA 132, and saw a slight reduction in number of unassigned reads, but found that the subsequent analyses were in agreement with Greengenes so we elected to keep the original assignment.

Principle Coordinate of Analysis (PCoA) plots and taxa bar plots were generated using the vegan package in R [11]. Profile clustering patterns from unweighted UniFrac distance measures were analysed using permuted adonis and betadisper tests. SourceTracker was applied to collapsed feature tables with macaques as source and humans as the sink to further characterize microbial sharing.

3. RESULTS

Demographics: All participants were born in Thailand and had lived in the Maha Sarakham province for over a year. Demographic factors are summarized in Table 1. Of note, participants in the exposed group were older than those in the control group and had fewer years of education. Among macaques, the controls sampled tended to be younger than those among the exposed population.

Table 1.

Demographic Factor		
Humans	Exposed (n=12)	Control (n=6)
Age, years (mean ± SD)	47.17 ± 11.36	27.5 ± 9.44
Sex		
Male	75% (9)	50% (3)
Female	25% (3)	50% (3)
Education, years (mean ± SD)	9.0 ± 3.05	16.8 ± 5.76 [1]
Household size		
1-3	25% (3)	67% (4)
4-6	58% (7)	33% (2)
7-9	17% (2)	0
Macaques	Exposed (n=8)	Control (n=4)
Age		
Juvenile	0	75% (3)
Subadult	37.5% (3)	0
Adult	62.5% (5)	25% (1)
Sex		
Male	50% (4)	50% (2) [1]
Female	50% (4)	25% (1) [1]
Group		
Red Dot	2	NA
Hare Lip	2	NA
Stump Tail	2	NA
Droop Lip	2	NA
Group 1	NA	4

[1] Missing n=1

Occupational Factors: Most of the exposed workers were government employees of Kosumpee Forest Park (n=8), followed by janitors at a nearby school (n=3), and a vendor stationed near the park entrance (n=1). On average, workers in the study had been at their current job for 18.40 ± 11.79 years (range: 0.25-41). Workers reported spending an average of 45.08 ± 8.694 hours per week (range: 33-63) around macaques and/or their feces, as part of their job. Two of the exposed participants also reported that they work on a farm.

Handwashing was primarily done using water only (n=6), soap and water (n=5), water only / soap and water (n=1) or water only / alcohol-based hand sanitizer (n=1). While all participants reported handwashing before and after eating, task observation footage suggested this is not the case for at least 4 participants. Few respondents reported using disposable gloves (n=1), paper dust masks (n=1), cloth masks (n=1), rubber/poly boots (n=3). PPE was largely not provided by the employer with one park worker reporting that cloth masks were available and used by that respondent, however none of the workers were seen wearing masks or gloves in video recorded task observations. Respondents did not report receiving training in animal behavior, animal capture/restraint, infectious disease prevention, PPE use, or wound care before working around macaques.

Five of the 12 workers noted changes in monkey behavior since they started working at their job, including that they are naughtier, wait for provisioning or do not look for natural food, and, in particular, several noted that they eat more human food like chicken, meatballs and soda. All workers reported finding monkeys that looked sick or had died, with frequency ranging from 2-3 times per year to 4-5 times per month. Carcasses were buried or burned. Typically, the monkey died from getting hit by a car, a dog bite, or from injuries in a fight with other monkeys. Two janitors working at the school said they ask forest park staff to pick up the carcasses, however one janitor remarked that, "Last month 3 monkeys die, pick them up by broom into plastic bag and then threw them into the forest."

Knowledge of Zoonoses: Four of the twelve workers (33%) knew that a diseased animal could transmit that agent to a human and two workers were unsure. The same number were concerned about this occurring from animals they may contact at work. Only one worker thought

a human could make an animal sick, and noted that this would be with a high degree of contact. This worker also reported that this might be a concern from animals at work.

All but two respondents (one worker, one control) believed that an animal that looked healthy could still be sick or were unsure. Ten of the 12 workers (83%) said they take extra precautions when working around animals that are possibly sick, primarily by avoiding contact (n=6).

Workers typically only have direct physical contact with carcasses, but reported trapping live monkeys to move them from private properties to the forest park or when helping researchers. In one instance, a janitor had to remove a monkey from a classroom using a stick and grabbing it by hand.

Health and Microbiome Factors: Health related factors among human exposed and control groups are summarized in Table 2. Among workers, the general disease concerns were recorded via a free response survey question and therefore open to diseases that they would not contract from exposure to macaques. Workers were primarily concerned about getting leptospirosis (n=3), cancer (n=2), the common cold (n=2), cirrhosis (n=1), allergies (n=1), and an airborne infectious disease (n=1). One worker was concerned about a "disease that come with monkey poo because I have to sweep it every day." In contrast, high blood pressure (n=3), cancer (n=1), diabetes (n=1) and hemorrhoids or constipation (n=1) were the main disease concerns among controls.

Table 2.

Health Factor	Exposed	Control
Fair	77% (8)	0
Good	33% (4)	83% (5)
Excellent	0	17% (1)
Smoker	75% (9)	0
Health problems in past year		
Fever	92% (11)	67% (4) [1]
Respiratory problems	58% (7)	67% (4) [1]
Gastrointestinal problems	33% (4)	67% (4)
Skin problems	25% (3)	0
Endocrine problems	17% (2) [2]	0 [1]
Infectious diseases in lifetime		
Tuberculosis	8% (1)	0
Malaria	8% (1)	0
Dengue	17% (2)	0
Typhoid	0	0
Other parasites, hookworm	58% (7) [1]	0 [1]

[1]Missing n=1; [2]Missing n=2

An abbreviated food frequency questionnaire revealed that one member of the control group reported consumption of unpasteurized dairy products 1-2 times per year, whereas no other participants reported consuming this item. Control group members also tended to consumed more pork, sugar sweetened beverages and artificially sweetened beverages. Exposed workers were more likely to report consumption of raw meat or fish, as seen in Table 3. All respondents reported that they pass normal formed stool (Type 3/4 on Bristol stool scale), except one, from the exposed group, who tends to have difficulty passing stool (Type 1/2).

Table 3.

Microbiome factor	Exposed	Control
Vaginal birth method	77% (8)	50% (3)
Primary infant diet		
Breast-fed	92% (11)	33% (2)
Mixture of breastmilk and formula	8% (1)	33% (2)
Formula	0	17% (1)
DK	0	17% (1)
BMI	25.5 ± 5.8	23.8 ± 3.5
Swim in natural water sources		
Never	50% (6)	50% (3)
1-2 times a year	33% (4)	33% (2)
1-2 times a month	17% (2)	0
1-4 times a week	0	17% (1)
Raw Meat/Fish Consumption		
Beef	67% (8)	33% (2)
Pork	8% (1)	0
Antibiotic use in past month	17% (2) [4]	33% (2) [2]

[2] Missing n=2; [4] Missing n=4

Early life factors are believed to play an important role in shaping the adult microbiome, and there were differences in delivery method and infant diet between exposed and control groups. More antibiotic use was reported by controls in the past month) were recorded [13].

Task Observation of Workers: Park workers, both cleaning staff and the designated monkey feeder, engaged in the highest exposure activities based on recorded task observations, followed by individuals working as school janitors, then vendors. Based on the risk rating scheme to assess exposure, park workers were 1.8 times as likely as school janitors and 2.5 times as likely as the vendor to contact macaques or engage in an activity that might facilitate microbe transmission during the task observation. Their high risk rating on the task observation is primarily due to the close proximity of macaques, with occasional direct contact, while they were engaging in aerosol generating activities and/or eating, drinking, or smoking. The high number of hand-to-mouth activities and work without respiratory protection represents a clear pathway

for transmission of microbes that may be present in aerosolized macaque feces and offers insight into possible risk mitigating interventions. Due to the small sample size, we elected not to use scores from task observation videos in microbiome composition analysis, instead treating all workers as exposed, however it is worth noting that the degree of exposure does indeed vary within this group.

Phylum-level Abundance: A total of 3,307 ASVs were generated from 628,623 total read counts. There was an average of 20,954 reads per sample (range: 12,466-35,318). Figure 1 shows the abundance of bacterial phyla in each sample, after rarefication to minimum sample size. All profiles were dominated by Bacteroidetes, Firmicutes, and Proteobacteria. Macaque samples differed from human samples in that they typically contained more Cyanobacteria, Tenericutes, Verrucomicrobia.

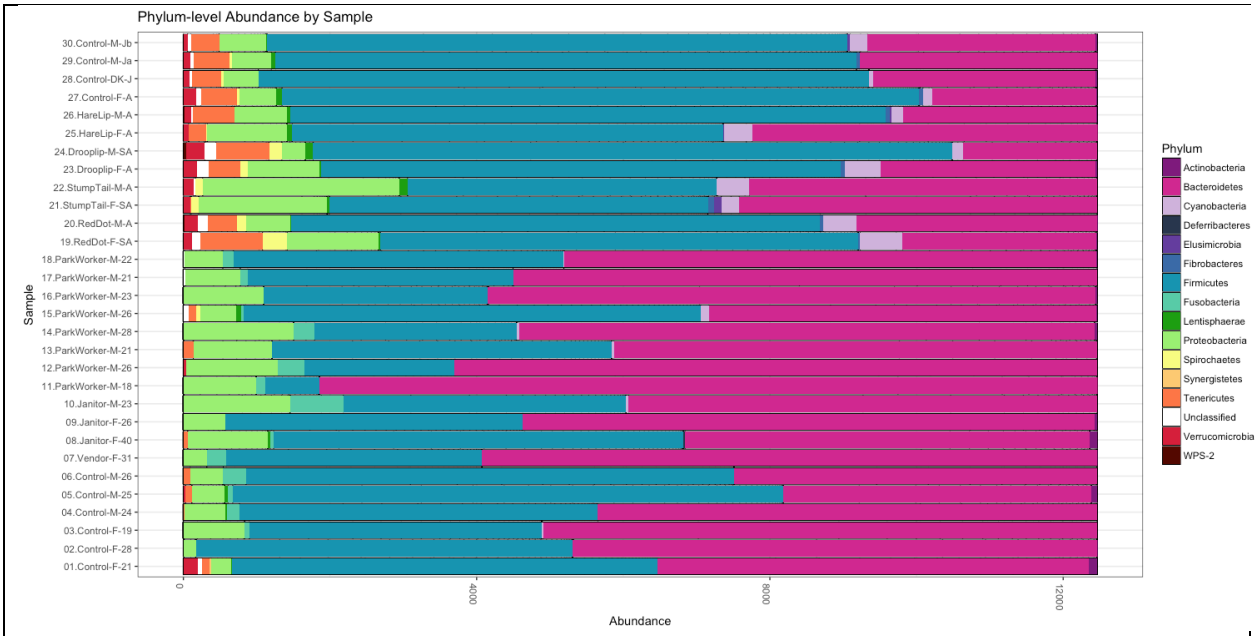

Figure 1. Abundance of bacterial phyla in macaque control (n=4), macaque exposed (n=8), human exposed (n=12), and human control (n=6) samples.

Alpha and Beta Diversity: Figure 2 displays the 1) total number of observed features in each sample, 2) Shannon's index, which accounts for abundance and evenness of the taxa present using a natural logarithm, and 3) Simpson's index, which measures the relative abundance of the different species making up the sample richness. Alpha diversity was significantly lower among exposed humans compared to controls using the Simpson index (Mann-Whitney, p=0.03), but not significantly different using other metrics. Control and exposed macaque alpha diversity levels did not differ significantly.

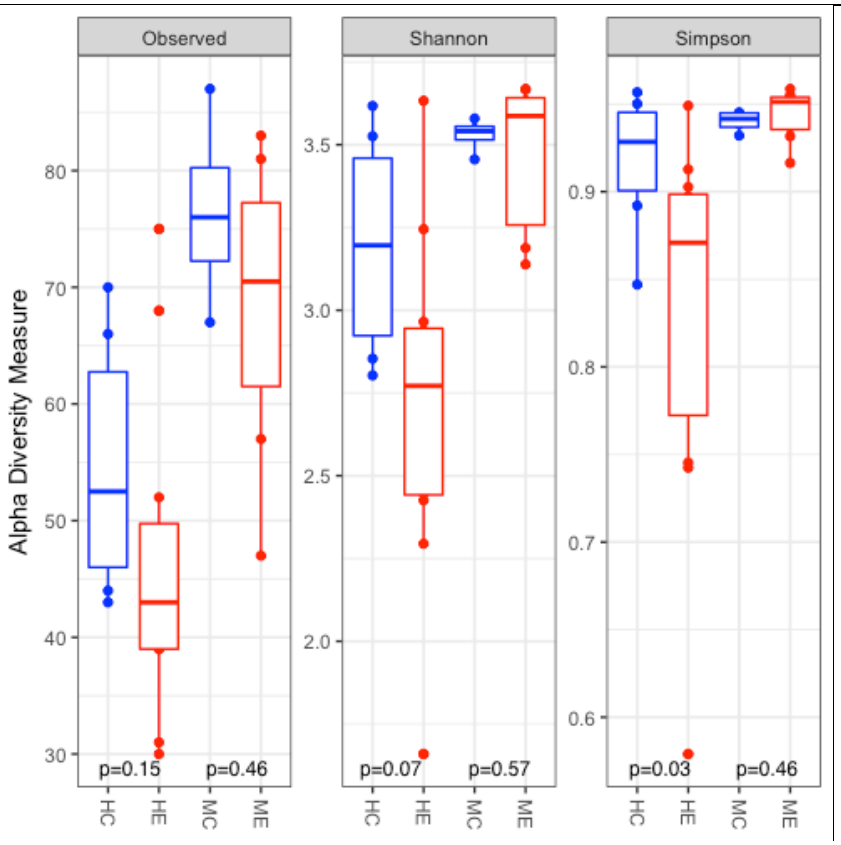

Figure 2. Observed taxonomic features, Shannon index, and Simpson index metrics for assessing alpha diversity were significantly lower among human exposed (HE) relative to human controls (HC) using the Simpson Index only. No statistically significant difference was detected among macaque exposed (ME) and macaque control (MC) groups.

Unweighted UniFrac PCoA plots were generated to visualize clustering patterns based on unweighted UniFrac distance measures, which describes the degree of similarity between sample

compositions by measuring the fraction of unique branch length from the phylogenetic tree of sample features (Figure 3). Dispersion using the betadisper test was significant for macaques (p=0.002), but not humans (p=0.357). When humans who reported taking antibiotics in the past month were excluded from the analysis (2 participants from control and 2 from exposed), the dispersion was found to be significant (p= 0.074). Adonis was used to test for location shift of the spatial median based on exposure status was significant for macaques and humans (p=0.05 and 0.002, respectively). It should be noted that, since the assumption of equal group variances is violated, this test is not technically valid for macaques, however, since it is the larger group with the greater dispersion, it is liable to be too conservative, therefore the significant shift in spatial medians likely holds [14]. All tests were performed using 999 permutations based on the spatial median in vegan [15]. Dispersion and location tests were also performed for weighted UniFrac, Bray-Curtis, and Jaccard distance measures, but the conclusions were unchanged.

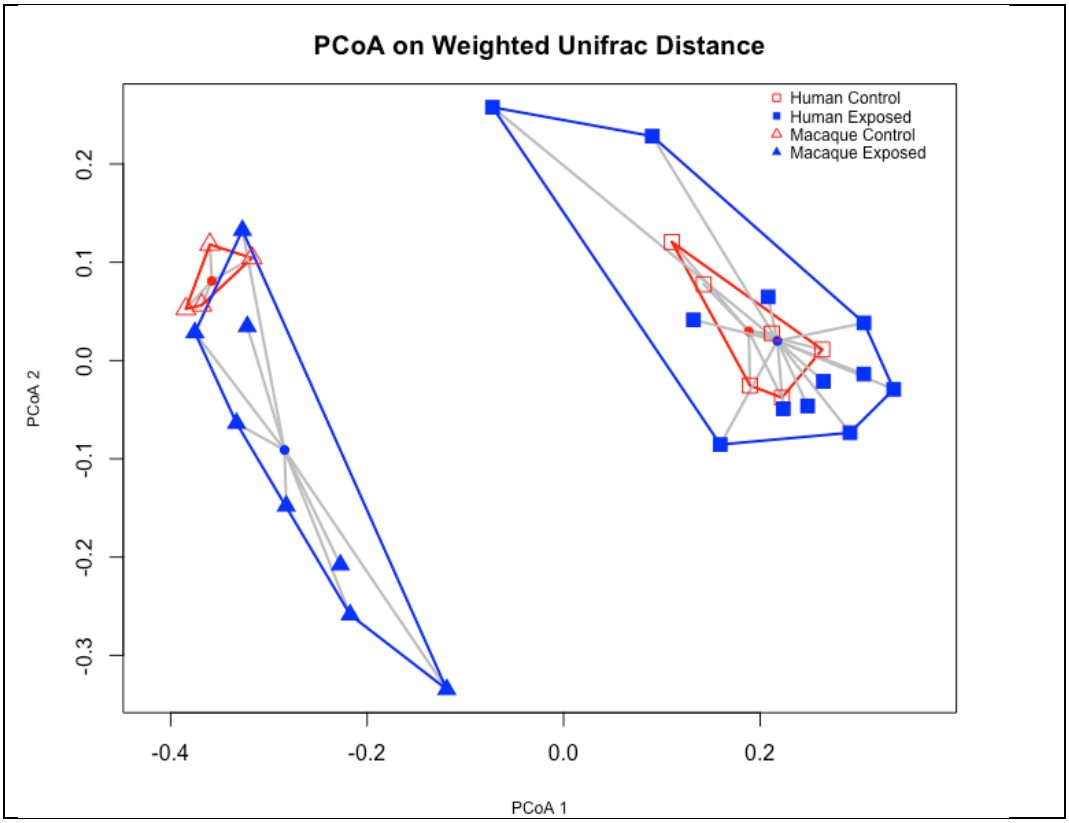

Figure 3. 2D PCoA plot based on unweighted UniFrac distances to demonstrate clustering and dispersion patterns for exposed human/macaque and control human/macaque samples.

Microbiome Sharing: SourceTracker was used to attribute possible sources of taxa identified in human exposed and control samples originating from macaques.[16] Percent of taxa in human samples attributable to these sources are expressed in Table 4. SourceTracker analysis revealed a greater percentage of microbes in the exposed human samples potentially sourced by macaque microbiota compared to the controls, however this difference is not significant.

	Source		
Sink	Macaque Control	Macaque Exposed	Unknown/Other
Human Control	5.85% ± 2.13%	3.90% ± 1.52%	90.25% ± 2.27%
Human Exposed	5.87 % ± 1.17%	7.31% ± 1.69%	86.82% ± 1.61%

Table 4. SourceTracker analysis of percent of taxa using Gibbs sampling with collapsed macaque samples as the sources and collapsed human samples as sinks.

Reads classified as belonging to *Succinivibrio* (genus), Treponema (genus), YS2 (order), and Streptophyta (order) were detected at higher levels in macaques and exposed humans compared to control human, but not found to be significant after adjusting for multiple comparisons (Figure S1 in Supplemental Materials). Based on the false discovery rate (FDR), only *Odoribacter* spp., *Holdemania* spp., *Bacteroides eggerthii*, and *B. uniformis* exhibited statistically significant differential abundance, however in each case, these taxa were enriched in human controls relative to the other groups, and therefore are not candidates for microbes shared between exposed humans and macaques.

4. DISCUSSION

The SourceTracker analysis suggested that there may be a baseline level of microbial sharing around five percent in both human populations, possibly mediated by common environmental sources. Likely due to the high degree of compositional variation in the exposed samples (e.g., significant dispersion), the percent of the taxa which was contributed by exposed macaques to control humans was actually lower than that of control macaques. However, among exposed humans, the estimated percent of taxa contributed by exposed macaques was almost double that of controls. While these values are not statistically different, it is possible that, with a larger sample size, we might detect a significant difference. With such a small sample size, we run into issues with beta error, and may fail to reject the null hypothesis due to insufficient power. This is a serious limitation that should be addressed in any future studies.

Our analysis revealed that workers exhibited a different composition of gut microbiome communities than controls, as evidenced by significantly different spatial medians in the PCoA of UniFrac distances. This finding may be due to a number of other exposure factors that warrant further investigation to determine the consequences of this location effect, including differences in age, SES, smoking status, delivery mode, and history of infectious diseases. While there is a considerable difference in age, all subjects were adults, so this factor alone is not expected to greatly influence results as gut microbiota, which tends to be well-established in healthy adults. Healthy adults' gut microbiomes are usually less sensitive to perturbations than infants, whose microbiota are developing and have not reached a stable state and elderly (>75 years old), who tend to have lower total bacterial levels [12]. However, the difference age may be related to other factors (e.g., infectious disease history), which could shift their microbial composition.

The macaques in the park have a high level of gut microbiome dispersion relative to the macaques with little human contact. Dispersion essentially reflects variation of microbiome composition, that is the taxa present and their abundance differs from sample to sample among exposed macaques, whereas the control macaques are composed of similar taxa at a similar level, and therefore cluster tightly together, with minimal dispersion. This significant dispersion pattern on exposed macaques is suggestive of the "Anna Karenina principle," a signature of dysbiosis characterized by increased variation in profiles of individuals in a disease state [17]. This dysbiosis may be due to environmental stressors or diseases that perturb a stable state in an unpredictable manner. We cannot definitively determine whether AKP effects are occurring without longitudinal sampling, however the initial findings are suggestive of these effects. In the KFP population, this dysbiosis could be a result of increased stress and competition among macaques, an increased disease burden, or may be attributable to their atypical diet of bananas. When asked if they noticed any changes in macaque behavior, workers reported that the macaques drank more Coca-Cola and ate more chicken than they used to. While most of the provisioned food appears to be fruits and veggies, according to RK, who has observed this population extensively, the more extreme dietary changes like foraging in trash, as noted in the Hare Lip group, might explain the high variation in composition among macaques at KFP. The population of macaques in this forest park and the adjacent village is also nearly 11 times denser than a typical free-roaming macaque population [2]. This results in elevated stress and aggression among macaques, which may ultimately facilitate pathogen spread. Given that their microbiota appear to be in a dysbiotic state relative to macaques with low levels of human contact, a condition that may predispose them to gut-related diseases, they might be expected to present a greater health threat to humans than wild macaques with traditional gut flora [18, 19].

One attribute of AKP effects, is that the stochastic variation in microbial composition makes it challenging to determine whether there is a certain set of taxa contributing to the observed spatial median shift. Indeed, we found that there were no specific taxa that were enriched in exposed populations, but did detect taxa that were significantly more abundant in the human controls. However, by characterizing these exposures and resulting gut microbial community shifts, we can leverage these insights to make informed recommendations to mitigate negative aspects of contact between humans and macaques and optimize the health of both populations.

Further research should 1) investigate temporal trends and the stability of the dysbiosis described in this study, 2) utilize a control population within Kosum Phisai with improved matching for confounding factors, and 3) incorporate testing for GI parasitism to improve understanding of the drivers of these microbial changes. The recommendation to test for GI parasitism and survey anti-helminthic drug use is based on the knowledge that members of this community take anti-helminthic medication prophylactically and the role of both these drugs and GI parasitism in altering gut microbiota warrants closer investigation. In particular, care should be taken to find well matched controls (e.g. matched age, SES, gender), to minimize the number of confounding factors in microbiota comparisons. If possible, these controls should come from the same village as the workers, in order to further reduce differences that are not related to their exposure to macaques.

While the threat of acquiring an infectious disease shed through macaque feces from their work tasks appears low, we recommend that basic PPE be used, such as closed toed shoes, to further reduce the risk from environmentally transmitted parasites, which can enter through the skin. One worker noted that they were experiencing respiratory issues, which they attributed to

the sweeping of monkey feces. Even if there were no microbial hazards from this exposure, the dust particles alone or endotoxin from Gram negative bacteria can cause irritation to the lungs. Spirometry or exhaled nitric oxide testing might help determine whether this work exposure is contributing to decreased lung function or increased inflammation, respectively. Use of a mask during such tasks or misting of the ground prior to sweeping may reduce exposure to aerosolized macaque feces and protect worker health.

5. CONCLUSIONS

This study draws on a One Health approach to reduce human-animal conflict in a setting modified by habitat encroachment (i.e. a condensed natural space in the forest park). Characterizing shifts in gut microbial communities allows for improved understanding of whether health changes are occurring due to increased human-macaque contact in a shared environment. By identifying opportunities for exposure and assessing whether transmission is occurring, we can inform recommendations for worker health and safety measures that address the health of both human and macaque populations. The primary goal of such measures is to reduce the risk of disease spillover from macaque populations into human communities based on targeted health protection and disease awareness promotion among forest park workers, who represent a bridge for transmission of diseases to the larger community.

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SUPPLEMENTAL MATERIALS

Score	Exposure	Example
0	Not applicable	Office work/indoors, worker has no contact with macaques other potentially infectious bodily fluids/excrement
1	Buffer, no direct contact	Worker is doing tasks in the vicinity of monkeys (>3m) or their bodily fluids/excrement but does not have an immediate risk of contact
2	Close proximity, no direct contact	Worker is doing tasks in close proximity (<3m) to monkeys but does not have any contact with monkeys or their bodily fluids/excrement
3	Brief contact w/ no injury	Monkey brushes against worker or takes banana directly from workers hand or direct contact with macaque bodily fluids/excrement
+1	Aerosol generating task	Sweeping grounds or driving over dirt roads where aerosolization of dust/dirt can occur without use of a respirator
+1	Hand to mouth contact	Eating/smoking/drinking in proximity of macaques or after contacting macaques/excrement and not washing hands

Table S1. Five minute segments from the task observation videos were scored based on macaque interaction as defined in the table above. The table has been truncated to only include activities/exposures that occurred in this study and a per-minute rating obtained by dividing the sum by the total task observation time. Activities like performing aerosol-generating tasks or hand to couth contact (smoking, eating) elevated the assigned risk score.

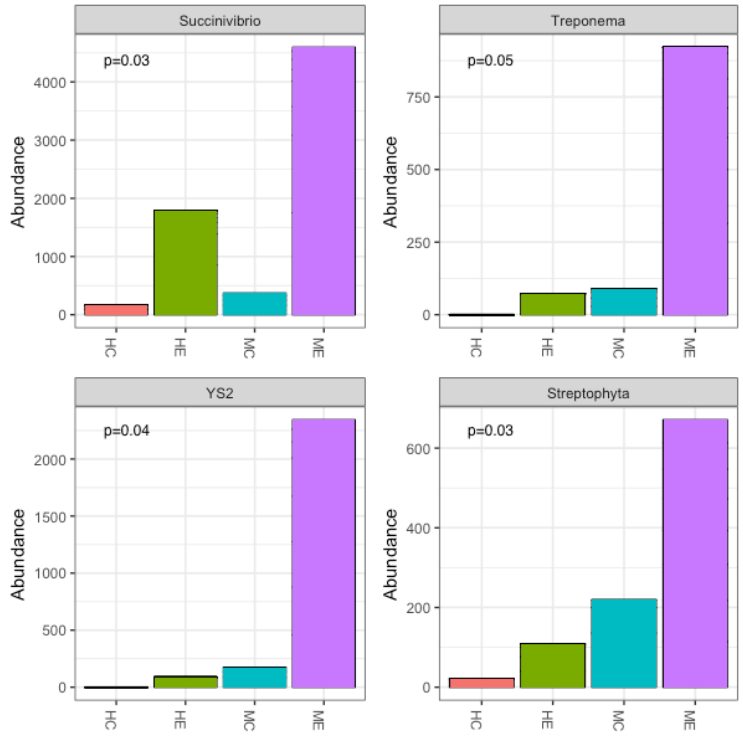

Figure S1. *Succinivibrio*, *Treponema*, YS2, and Streptophyta were enriched in macaque and exposed human populations. However the significance of this difference did not hold after adjustment for multiple comparisons using FDR.