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Analysis of Wild Rodent Gut Microbiota as a Function of Exposure to Ticks and Tick-borne Pathogens

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by

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Abstract

Due to advances in high-throughput parallel sequencing, researchers have conducted novel studies exploring relationships between microbiome compositions and different aspects of organism health. Some of these studies have shown that the gut microbiome of rodent models has effects on organism health and behavior and that infection with pathogens and the composition of the skin microbiome are linked to changes in gut microbiome composition. While previous studies have shown how vector microbiota impact vector behavior and pathogen transmission, the effect vectors have on reservoir species microbiomes has been a less prominent focus. We were interested in the relationships between tick parasitism, tick-borne pathogens, and host rodent microbiomes. As ticks interact with the skin, it is reasonable to believe tick parasitism can affect the host skin microbiome, which can affect the gut microbiome and overall rodent health. Additionally, tick-borne pathogens could possibly affect the skin and gut microbiome, resulting in health or behavioral changes. However, it is difficult to determine if tick parasitism and pathogens are affecting the host microbiome, or if certain genetic, behavioral, and microbiome factors in the host promote tick parasitism and pathogen susceptibility. To determine what relationships were present in this system, we first sampled two species of rodents (*Peromyscus leucopus* and *Sigmodon hispidus).* We detected significant variation in gut microbiota among species, but not between sampling sites. Moreover, we found gut microbiome composition, as well as microbial richness, to vary as a function of tick parasitism and infection with the Lyme disease agent, *Borrelia burgdorferi*, although we note that some of these differences were species-specific. Based on the results of this study, we performed a follow up experimental study on lab mice in which we collected gut microbiome data before and after *Ixodes scapularis* tick parasitism. Our preliminary analysis detects that both tick parasitism and the origin of the ticks used (coastal or mountain) could influence rodent microbiome composition.

Introduction

Advances in high-throughput parallel sequencing have allowed for novel exploration of the drivers of microbial diversity within and outside an organism (1). These advances have given rise to several recent studies linking microbiome compositions to overall organism health and behavior. Specifically, studies have correlated microbial species composition to processes related to development (2), neurological disease (3), and behavioral responses to stress (4).

The use of rodent models of human disease has provided a wealth of information about links between gut microbiota and a variety of health outcomes including endocrine signaling (5) gastrointestinal dysfunction (6, 7) and depression (8). Some studies shed light on specific mechanistic drivers of microbiome on organismal health. In one study, mice became obese and developed insulin resistance upon dysbiosis of the gut microbiome, but insulin production rebounded and weight loss occurred when gut bacteria were induced to release more acetate (9).

While much of the work related to gut microbiomes has focused on chronic conditions, there is a growing area of research focused on the relationships between gut microbiome composition, infectious diseases, and the immune system (reviewed in 10). Round and Mazmanian (11) reviewed studies of mouse models and humans and concluded that elements of the microbiome play a key role in protecting the host from potential pathogens either through immune system modulation or production of metabolites that reduce the likelihood of infection.

More recent studies have linked changes in human microbiome composition development of sequalae from SARS-CoV-2 infection (12) and Lyme disease (13).

These previous studies demonstrate that the gut microbiome is related to overall organism health and behavior, and additional studies reveal that the gut microbiome and skin microbiome are linked via a skin-gut axis (reviewed in 14). Studies have shown that gut dysbiosis has been linked with inflammatory diseases of the skin, and some food ingredients can impair the intestinal barrier and allow gut bacteria to enter the bloodstream, which can affect other parts of an organism, including the skin (14). Just as the gut microbiome can influence the skin, the skin microbiome can influence the gut. Studies have shown that skin exposure to UV light can increase bacterial diversity in the gut microbiome (14). From these studies, we now know that the skin microbiome, gut microbiome, and overall organism health and behavior are related (Figure 1).

Microbiota can have unique and specific impacts on vector-borne infections. Recent studies have emphasized the role of the vector microbiome in transmission of vector-borne zoonoses (15-17), and manipulation of the vector microbiome may yield new mechanisms to control these diseases (18). For example, the microbiome composition of mosquitos has been linked to vector competence for diseases such as Dengue and West Nile (19), while the microbiome of ticks affects the competency to spread *Borrelia burgdorferi*, the causative agent of Lyme Disease (20). Host bloodmeal source has also been shown to affect the microbiome and competence of vector species (21, 22). These studies demonstrate how vector microbiota impact vector behavior and pathogen transmission, but the influences vectors and vector-borne pathogens have on reservoir species microbiomes has been a less prominent focus. We were interested in exploring the relationships between tick parasitism and tick-borne pathogens on

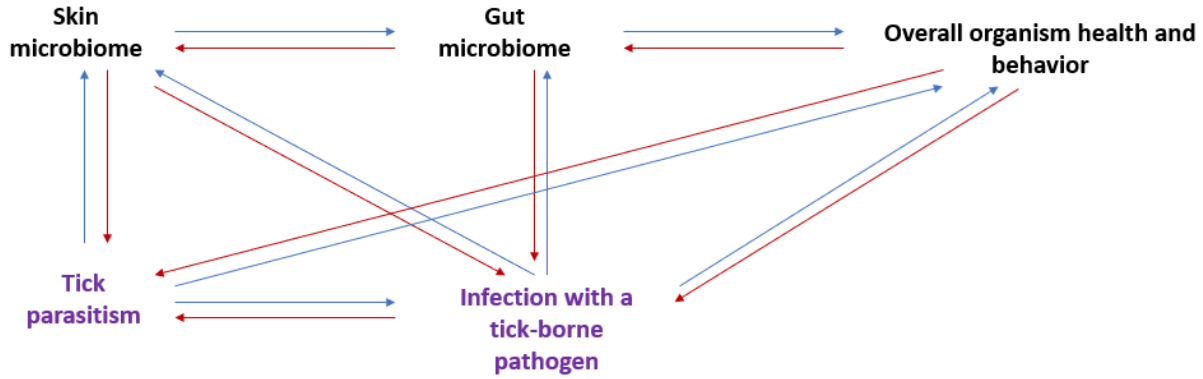

Figure 1: Possible interactions tick parasitism and infection with a tick-borne pathogen could have with rodent microbiomes and overall organism health and behavior*. Previous studies have shown that the skin microbiome and gut microbiome are linked, and the gut microbiome has been linked to changes in organism health and behavior. As ticks interact with rodent skin, it is reasonable to expect tick parasitism to affect the skin microbiome, which affects the gut microbiome, and thus organism health and behavior. Additionally, tick parasitism could directly cause changes in organism health and behavior through a pathway independent of the microbiome. Tick-borne pathogens could possibly induce changes in the skin or gut microbiome, which can affect organism health and behavior, and tick-borne pathogens can also induce changes in organism health and behavior in a pathway independent of rodent microbiomes. We cannot say with certainty the direction of causation. An organism with certain health and behaviors due to genetic factors, can potentially promote certain skin and gut microbiomes, which affects the likelihood of tick parasitism and infection with a tick-borne pathogen. Additionally, certain genetic factors can predispose a rodent to become tick parasitized or infected with tick-borne pathogens independent of microbiome composition.*

host rodent microbiomes and determining how these relationships fit into the previously established relationships between the skin microbiome, gut microbiome, pathogens, and overall health and behavior.

As ticks primarily interact with the rodent skin, we proposed that tick parasitism could affect skin microbiome, which could affect the gut microbiome and overall health and behavior. Additionally, tick-borne pathogens could potentially affect the skin-microbiome and gut-microbiome, which could lead to changes in overall organism health. However, we cannot assume that these pathways are unidirectional. Perhaps certain individual rodents have genetics that predispose them to certain behaviors that results in greater tick parasitism or likelihood for disease. Some individuals may have a gut microbiome that predisposes them to be more susceptible to pathogens, or they may have a gut microbiome that affects the skin microbiome in such a way as to make tick parasitism more likely. It is difficult to determine if a rodent microbiome affects rates of parasitism and pathogen infection, if tick parasitism and tick-borne diseases affect the microbiomes and organism behavior and health, or if there are bidirectional affects at play (Figure 1).

In order to gain a better understanding of the possible interactions between rodent microbiomes, tick parasitism, and tick-borne pathogens, we performed a sampling study of two rodent species in central Virginia: the white-footed deer mouse, *Peromyscus leucopus*, which is a highly competent reservoir for the Lyme disease bacterium *Borrelia burgdorferi* (23), and the cotton rat, *Sigmodon hispidus*, which is a potential reservoir for the agent of Tidewater spotted fever, *Rickettsia parkeri* (24, 25). We also opportunistically collected intestine samples from brown rats (*Rattus norvegicus*) in Richmond, Virginia, that were being sampled for other purposes. We collected parasitism and tick-borne infection status of these rodents and performed a gut microbiome analysis of each sample using high-throughput parallel sequencing. Based on the relationships we found in this study, we performed a follow up experimental study that explored how the gut microbiome composition of lab mice (*Mus musculus*) is altered when parasitized by *Ixodes scapularis* ticks form two regions of Virginia (mountain and coastal). We expected that if exposure to ticks and tick-borne pathogens imposes physiological stress on host individuals, then the gut microbiome would be varied as a function of these factors as well.

Methods

Study #1: Sampling Rodents in Central Virginia

We sampled rodents from May through July 2020 at two sites in central Virginia; Ft. Pickett, at the intersection of Nottaway, Lunenburg and Dinwiddie Counties, and a site in Goochland County owned by the University of Virginia. We sampled early successional open-canopy habitats at each site where we set eight transects of 10 traps with 10 m spacing between traps and 150-1500 m between transects. Traps were baited with bird seed mixed with a small amount of apple cider and set for three consecutive nights, once every three weeks. Traps were typically checked by 10:30 each morning and locked open, but not set, between sampling sessions. In addition to applying individually-numbered ear tags, identifying rodents to species and collecting information on sex, we collected a ~ 1 mm2 tissue biopsy from the ear and any ectoparasites from the head, ventrum, or limbs. We collected feces into 1.5 ml microcentrifuge tubes while rodents were in-hand. Fecal samples were frozen dry at -20 C whereas ectoparasites and ear punch biopsies were stored in 70% ethanol at -20 C prior to DNA extraction. Any individuals that died in traps were held on ice until they could be stored at -20 C. These samples

were thawed and dissected in spring 2021 so that roughly 0.5 cm of small intestine, taken from roughly halfway between the stomach and colon, could be collected for microbiome analysis. In late 2021, we obtained additional rodent carcasses from sampling in southeastern Virginia; these were dissected and small intestine samples were removed as described above. All animal work was reviewed and approved by the Institutional Animal Care and Use Committee of the University of Richmond (protocols 20-01-001 and 20-06-002).

Study #2: Tick-exposure experiment

We ordered 10, seven-week-old female lab mice (*Mus musculus*). The mice were kept indoors for seven days and fed food and water until the experiment began. Before beginning the experiment, a fecal sample was collected from each rodent. The mice were split into two random groups of five individuals each. One group was randomly assigned northern (mountain) *Ixodes scapularis* ticks and the other group was assigned southern (coastal) *Ixodes scapularis* ticks. Ticks were in the larval stage and free of pathogens. They originated from eggs of engorged females that were collected in fall 2021 from hunter-killed deer. The mice were transferred into outdoor cages lined with dry, tick-free leaf litter for 12 hours. During this period, a known number of ticks (20-50) were introduced into the cage and allowed to feed on the mice. After this period was over, the mice were transferred back inside where they were kept for seven days. Over the course of 3-5 days, engorged ticks dropped off the mice and were collected. Fecal samples were collected following this seven-day period. All animal work was reviewed and approved by the Institutional Animal Care and Use Committee of the University of Richmond (protocols 20-01-001 and 20-06-002).

Molecular methods

DNA was extracted from fecal samples and ear punch biopsies using a commercial kit (Macherey-Nagel Blood and Tissue Kit, Macherey-Nagel, Inc. Bethlehem, PA) and following manufacturer's protocols. Tissue DNA was subject to polymerase chain reaction detection of *Rickettsia parkeri* and *B. burgdorferi*. Briefly, we screened each sample for *R. parkeri* by targeting the ompB gene using primers Rpa129R and Rpa224R along with probe Rpa188 and published reaction and thermocycler conditions (26). *B. burgdorferi* was detected using nested PCR and gel electrophoresis of the recG gene (primers and reaction conditions found in 27) and a probe-based qPCR of the oppA2 gene (primers and reaction conditions found in 28).

For microbiome analysis, we used primers 515F and 806R (29), modified by adding overhang sequences compatible with Illumina Nextera adapters (Illumina, Inc., San Diego, CA), to target a ~290bp region of the hypervariable V4 region of the bacterial 16S rRNA gene. Following initial PCR, amplicons were purified of remaining PCR reagents using AMPure magnetic beads (Beckman Coulter Life Sciences, Indianapolis, IN). Illumina Nextera adapters, containing unique barcode sequences, were then added (Illumina, Inc., San Diego, CA). Following a second purification step, library concentrations were measured with a Qubit fluorometer (ThermoFisher, Waltham, MA), diluted to 10 nM, and pooled together, prior to a final dilution to 150 pM. We added 5 ul of 100pM PhiX positive control library to 15 ul of the pooled library and sequenced from these samples on an Illumina iSeq.

Informatic and statistical analyses

Informatic analyses were conducted using QIIME2 (30). After importing demultiplexed fastq files, we assembled forward and reverse reads and sequence variants identified using the DADA2 algorithm (31) implemented in QIIME2. We note that we reduced minimum overlap between forward and reverse reads to 5 nucleotides to account for the 151 bp read length produced by the iSeq. Taxonomic assignments for each sequence variant were made using the GreenGenes database (version 13-8) in QIIME2 and this package was also used to analyze alpha and beta diversity. Statistical analyses of microbial community similarity were done by perMANOVA analysis, implemented in the R package vegan (32), and t-tests and ANOVAs to compare taxonomic richness and diversity among sample groups were also implemented in R (www.r-project.org). Changes in taxon abundance were tested with ANCOM-BC (33).

Results and Discussion: Study #1

Samples Collected and Tick Parasitism and Tick-borne Pathogen Status

We collected and sequenced fecal samples from 40 individual *Peromyscus leucopus* and 10 *Sigmodon hispidus* trapped at the two field sites in central Virginia (Table S1). Of these individuals, four (three *P. leucopus* and two *S. hispidus*) were recaptured but found dead in their traps on a subsequent trapping session and were subject to dissection and sequencing from a portion of the small intestine. These samples were supplemented by 11 *Rattus norvegicus,* collected from Richmond, Virginia, and two additional *P. leucopus* sampled from Chesapeake, Virginia (Table S1). Eight of 42 *P. leucopus* and one of ten *S. hispidus* tested positive for *B. burgdorferi* by PCR and no samples tested positive for *R. parkeri* by PCR. Of the 50 *P. leucopus* and *S. hispidus* individuals sampled in central Virginia, 18 were found to be parasitized by at least one tick. As there were no *Rattus norvegicus* samples that were tick parasitized or tested positive for *B. burgdorferi* or *R. parkeria,* we only conducted a preliminary analysis of these samples.

Reads and OTUs

Samples provided an average of 26,058 sequence reads (range 6,212-63,509) after quality filtering with a median read length of 290 base pairs; read depth did not vary as a function of sample type (gut versus feces; $T = 1.84$, $P = 0.07$). We detected 21,811 unique sequence variants which were classified into 425 operational taxonomic units (OTUs). Rarefaction analysis indicated no substantial increase in either observed OTUs or phylogenetic diversity after approximately 4000 sequences had been analyzed, suggesting a reasonable estimate of microbiome composition is possible with a minimum of roughly 5,000 sequences (Figure 2). OTU richness across all samples increased significantly with total sequence reads per sample ($F = 14.6$, $P < 0.001$, $R^2 = 0.54$), but Shannon's diversity index did not ($F = 2.48$, $P = 0.12$, $R^2 = 0.06$).

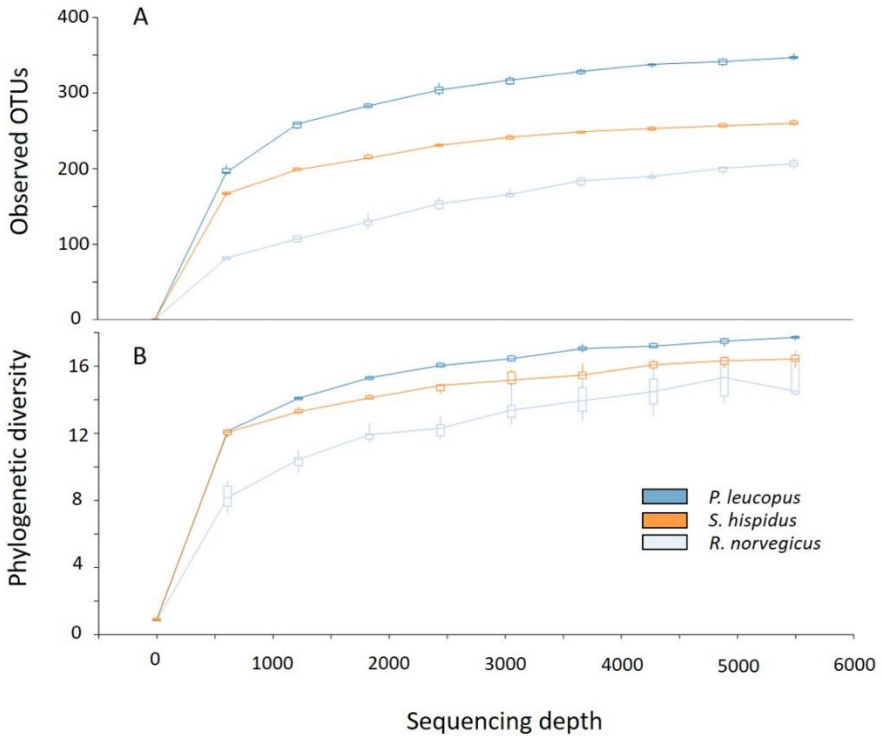

Figure 2: *Rarefaction analysis showing (A) accumulation of observed OTUs and (B) Faith's phylogenetic diversity as a function of sequencing depth per sample and shown for all three rodent species analyzed in this study. Rarefaction analysis indicated no substantial increase in either observed OTUs or phylogenetic diversity after approximately 4000 sequences had been analyzed, suggesting a reasonable estimate of microbiome composition is possible with a minimum of roughly 5,000 sequences*

Microbial Community Structure

Beta Diversity

Overall, gut microbiome composition varied significantly by species whether fecal (F=3.29, P = 0.008) or intestinal (F = 3.11, P < 0.001) gut data were analyzed (Figure 3A and 3B). Specifically, we found that the genera *Desulfovibrio* and *Helicobacter* differed in relative abundance by rodent genus (F = 5.12, P = 0.09 and F = 56.6, P < 0.0001 for *Desulfovibrio* and *Helicobacter*, respectively; Figure 1S). We note that for the intestinal data, we cannot separate the effect of sampling site from species because not all species were present at every site, and, moreover, the number of intestinal samples was relatively small. However, in the case of fecal samples, where we could test for site and species effects within the same model, there was no significant effect of sampling site on microbial community structure after accounting for variation attributable to rodent species (F = 0.35, P = 0.93).

A comparison of sample type (fecal versus intestine) from all individuals at the same field site demonstrated that for both *P. leucopus* (F = 5.45, P = 0.007) and *S. hispidus* (F = 12.42, P = 0.028) the fecal and intestinal microbiota were structurally different.

P. leucopus fecal microbiome structure did not vary by *B. burgdorferi* infection status (F = 1.32, P = 0.24) or by parasitism by ticks (F = 1.13, P = 0.32). The fecal microbiota of *S. hispidus*, however, varied weakly depending on whether the host individual was parasitized by

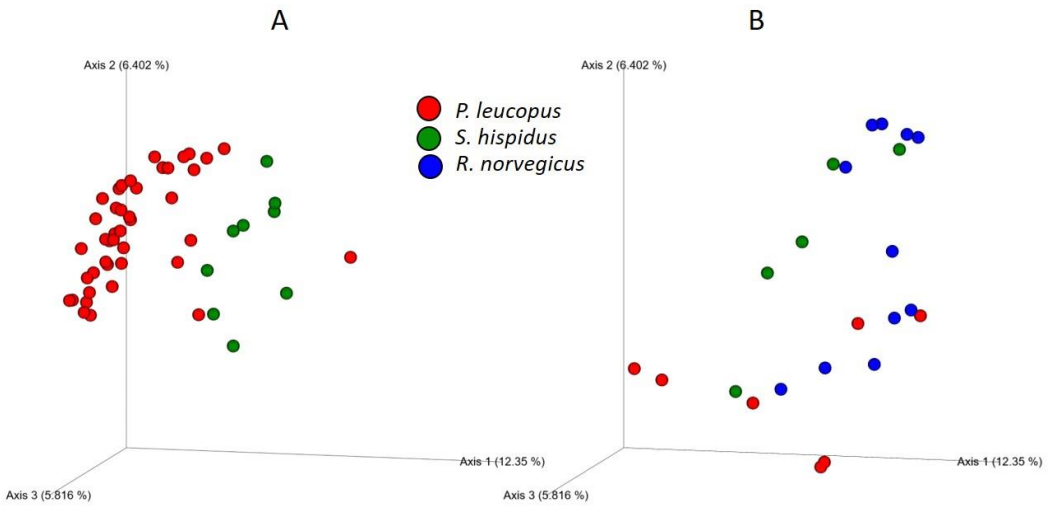

Figure 3: *UniFrac plots representing unweighted UniFrac distances showing relative variation in microbiome composition for fecal (A) and intestinal (B) samples from each of the three rodent species analyzed in this study. Both plots represent the same UniFrac analysis with different samples made visible to facilitate comparisons among species. The X, Y, and Z axes represent 12.4%, 6.4%, and 5.8% of the variation in this dataset, respectively. These plots are a visual representation of how the gut microbiome composition varied significantly amongst our three rodent species.*

ticks (F = 3.26, P = 0.052). Only one *S. hispidus* tested positive for *B. burgdorferi* so we were not able to test for differences in gut microbiome as a function of infection with this tick-borne pathogen.

Richness and Alpha Diversity

Across all *P. leucopus* and *S. hispidus* fecal samples, OTU richness was higher (F = 6.98, P = 0.01) in intestinal than in fecal samples but did not vary by host species (F = 0.972, P = 0.33). OTU diversity was not affected by sample type (F = 0.321, P = 0.57) or species (F = 0.271, P = 0.605). Analysis of OTU richness in fecal samples revealed a significant effect of tick parasitism (F = 4.1, P = 0.049), and tick-by-species interaction (F = 4.87, P = 0.032), but no effect of host species alone (F = 0.69, P = 0.41)(Figure 4). Tick parasitism had a similar effect on Shannon's diversity index by itself (F = 11.47, P = 0.001) and through interaction with host species (F = 5.21, P = 0.027) but there was again no difference in OTU diversity solely as a function of host species (F = 0.20, P = 0.162)(Figure 4). In particular, the bacterial family Muribaculaceae (formerly S24-7; Order Bacteroidales) was significantly more abundant in tick-parasitized than in non-parasitized *S. hispidus* (T = 3.49, P = 0.010), though no difference in relative abundance was detected in *P. leucopus* as a function of tick parasitism (T = 1.3, P = 0.20). Analysis of fecal microbiota indicated that infection of *P. leucopus* with *B. burgdorferi* had a significant positive impact on gut OTU richness (F = 16.7, P < 0.001) but no effect on diversity (F = 01.84, P = 0.18).

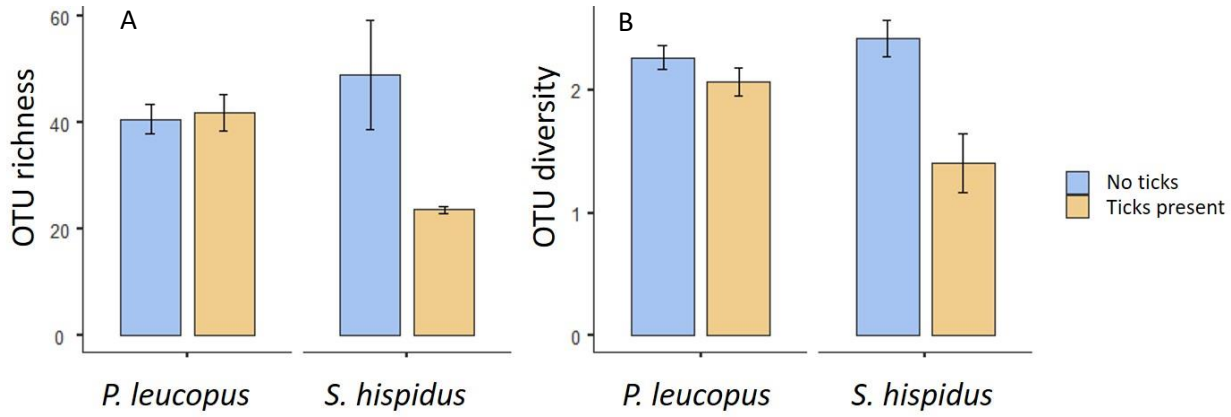

Figure 4: *Variation in OTU richness (A) and diversity (B) for both P. leucopus and S. hispidus as a function of tick parasitism. S. hispidus had significantly lower richness and diversity when parasitized, but there was not a significant difference for richness or diversity for P. leucopus between the parasitized and uparasitized groups.*

Discussion

The purpose of this study was to understand the different possible relationships that tick parasitism and tick-borne pathogens have with rodent gut microbiomes. We found significant differences in the beta diversity of the gut microbiome across three common rodent species from the southeastern United States, including the invasive brown rat. Additionally, OTU richness and alpha diversity did not vary by host species. These findings suggest that while the three species of rodent contain different taxa of bacteria in the gut microbiome, the number of different bacterial taxa and their relative abundances is similar amongst the three species. Additionally, our results show us that fecal and intestinal samples yielded different microbial taxa, which is consistent with previous studies (34).

With respect to the relationships of tick-borne infections and fecal microbiota, we observed fecal microbiota from *B. burgdorferi*-infected *P. leucopus* were significantly richer than those from uninfected individuals. We cannot rule out the possibility that this variation is driven by the effects of stochastic variation in sequencing output rather than *B. burgdorferi* infection itself, given that OTU richness varied as a function of the total number of sequence reads. In addition, OTU diversity did not differ significantly between infected and uninfected mice, suggesting that variation in richness was driven by infrequently-detected, low-abundance OTUs.

However, given that the tick-borne pathogen *B. burgdorferi* relies on its host and vector for a variety of metabolically important compounds it cannot synthesize itself (35,36), it is reasonable to speculate that the presence of *B. burgdorferi* may induce changes in the host metabolism and microbiome to promote the production of compounds it needs for survival. Currently unpublished research by Dr. Nicole Baumgarth at John's Hopkins seems to also suggest that the presence of *B. burgdorferi* can induce changes in rodent host microbiomes (37). While our results may be attributed to stochastic variation, it is possible that there is a relation between *B. burgdorferi* infection and rodent gut microbiomes that we cannot clearly observe due to uncontrollable factors that are inherent to a sampling study.

With respect to effects of ectoparasites on fecal microbiota, we observed a significant negative effect of tick parasitism on gut microbial richness and diversity for *S. hispidus*, but not

for *P. leucopus*, which could not be attributed to the effect of host species alone (Figure 4). Specifically, we observed that the bacterial family Muribaculaceae (Order Baceriodales), previously known as S24-7, was more abundant in tick-parasitized *S. hispidus*. Kreisinger et al. (38) reported increases in the abundance of this taxa in the presence of tapeworms in the intestines of wild *Apodemus flavicollis* mice. This taxon is believed to be important in carbohydrate metabolism (39,40) and recent studies have suggested that the presence of this taxon can compete with *C. difficile* for carbohydrate resources, thus making it difficult for *C. difficile* to colonize a gut microbiome (41). Based on these studies, we theorize that an increase in Muribaculaceae abundance is a common response among rodents in response to parasitism, and this result may have evolved to protect a parasitized host from developing an infection, such as one caused by *C. difficile.*

These sampling results provided us with a better understanding of how the microbiome of rodent microbiomes interact with ticks and tick-borne pathogens. However, these results do not show us cause and effect. We cannot determine if tick parasitism or pathogen infection induced microbiome changes or vice versa. Our data comes from a single time-point, so we cannot determine what our sampled rodents experienced before and after the sample was collected. Thus, there are factors that could affect the rodent gut microbiome that we cannot control due to the nature of sampling. Since we observed that tick parasitism can affect the gut microbiome, we followed up this study with a controlled experimental study that would allow us to collect fecal samples before and after rodents were parasitized with ticks.

Results and Discussion: Study #2

The analysis of the results from study #2 are still ongoing, so we present a selection of preliminary results.

Results

We collected and sequenced fecal samples from 10 female *Mus musculus* lab mice before and after parasitism with *Ixodes scapularis* ticks, for a total of 24 fecal samples. Mice were randomly assigned into two groups of five, and each group was exposed to either northern (mountain) ticks or southern (coastal) ticks. Tick parasitism ranged from 3-18 engorged ticks per mouse. There was significantly more attachment and engorgement of the mountain larvae.

When comparing before and after parasitism microbiome composition, we find that richness was significantly higher in samples prior to parasitism ($P=0.04$), but Shannon's diversity index did not change in response to parasitism ($P=0.83$). These results can qualitatively be visualized via a UniFrac plot (Figure 5). In these plots, the closer the sample points are in three-dimensional space, the more similar their microbiomes are.

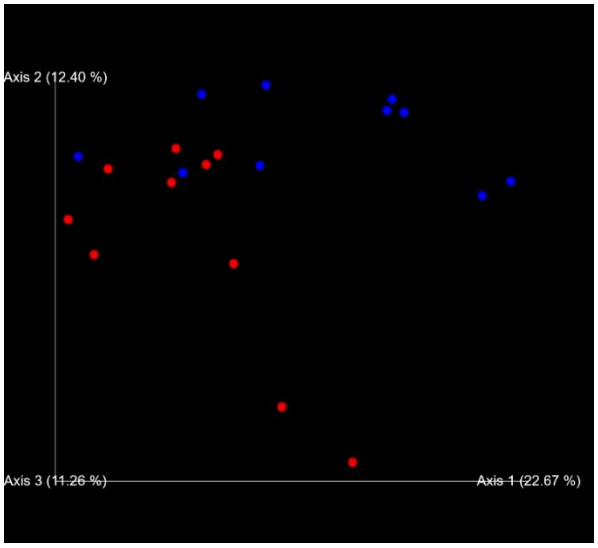

***Figure 5**: UniFrac plot representing unweighted UniFrac distances showing relative variation in microbiome composition for before parasitism (red) and after parasitism (blue) samples. The X, Y, and Z axes represent 22.67%, 12.40%, and 11.26% of the variation in this dataset, respectively. The before samples and after samples appear to form two separate clusters.*

After adjusting for multiple comparisons, ANCOM-BC identified seven taxa that changed significantly in relative abundance in response to tick parasitism: *Marvinbryantia*, Prevotellaceae UGC-001, Christensenellaceae_R-7 group, Lactococcus, Lachnospiraceae NK4B4 group, Muribaculaceae, and Rikenellaceae RC9 gut group. Each of these taxa increased in relative abundance following tick parasitism.

The origin of the tick involved in parasitism also affected the shift in microbiome composition before and after parasitism. Whereas southern coastal ticks had a significant change in richness (P=0.02), northern mountain ticks did not significantly change in richness in response to tick parasitism (P=0.72). These results can also be qualitatively visualized via a UniFrac plot (Figure 6).

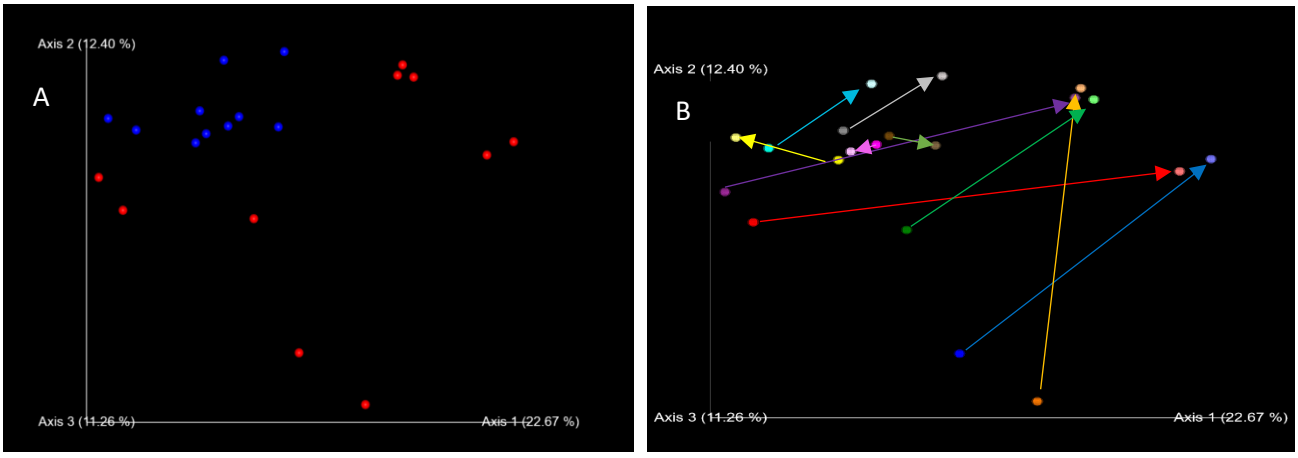

***Figure 6:** (A) UniFrac plot representing unweighted unifrac distances showing relative variation in microbiome composition for southern coastal tick-parasitized mice (red) and northern-mountain tick-prasitized mice (blue). (B) Each individual mouse before and after sample color-coded with an arrow drawn from before to after point. In tandem with 6A, this graph shows that northern ticks induced a relatively smaller microbiome change than southern ticks. The X, Y, and Z axes represent 22.67%, 12.40%, and 11.26% of the variation in this dataset, respectively.*

Discussion

The purpose of this study was to build upon results from study one by determining how before tick parasitism gut microbiome composition compares with after parasitism composition. From our preliminary analysis of the data, we can see that before parasitism and after parasitism samples make two different clusters on our UniFrac plots, which suggests that tick parasitism induces changes in the gut microbiome. Specifically, richness was higher prior to tick parasitism, but Shannon's diversity did not change. In other words, the number of different taxa present in the gut microbiome decreased upon exposure to ticks, but the overall relative abundances of taxa did not change. This suggests that less abundant taxa may be driving the richness changes we observed. Subsequent PERMANOVA analyses are needed to better elucidate the effects tick parasitism has on rodent gut microbiomes.

Our ANCOM-BC results show us that Muribaculaceae increases in abundance following tick parasitism. In study #1, we observed a similar change for the rat species *S. hispidus*. The similar change in abundance of this taxon among two different rodent species in response to parasitism gives further support in our hypothesis that Muribaculaceae commonly increases in abundance in response to parasitism. Other taxa that increased in relative abundance were Lachnospiraceae (containing the genus *Marvinbryantia*) and Rikenellaceae, both of which are involved in monosaccharide breakdown similar to Muribaculaceae (41). From these results, there appears to be a trend for a variety of monosaccharide consuming bacteria to increase in abundance upon the host species undergoing stress, such as tick-parasitism.

Our results also suggest that the origin of the tick involved in parasitism affects the changes in richness upon parasitism. Southern coastal ticks had a significant change in richness, whereas northern mountain ticks did not have a significant change in richness. From the results of this study, we know that northern ticks engorged at a significantly higher frequency than southern ticks. In other words, the northern ticks are more aggressive when it comes to questing and feeding behavior. We hypothesize that the northern ticks may have evolved to induce a smaller change in the host microbiome in order to prevent the host species from developing negative health effects, which could affect host foraging patterns. As a result, host species can continue to maintain behaviors in which they interact with ticks, thus giving the ticks more opportunities to feed. Southern ticks have other food sources, such as lizards (42), and thus there were fewer environmental pressures to select for ticks that induced small changes in rodent microbiomes.

As with study #1, there are limitations to study #2. The study lacks a control group of mice that remained unparasitized. This makes it difficult to determine the extent to which tick-parasitism causes the changes we observe. For example, some microbiome changes could have been induced by bringing the mice outside during the experiment. In addition, while this study allows us to more clearly see cause and effect compared to the sampling study, this study gives up using wild rodents in order to have more standardized results. Thus, these results may not perfectly match with the actual phenomena that occur in wild rodent populations.

Ongoing and Future Studies

One of the issues with study #2 is that there was no unparasitized control group. Thus, it is difficult to separate the effects tick parasitism has on the mice microbiomes from the environmental effects of moving the mice from indoors to outdoors. In our current study, we

obtained ten lab mice and kept them indoors over the course of the study. Five of the mice were randomly assigned to be parasitized by ticks and the other five were assigned as control groups. We gave the mice one week to become acclimatized to the environment before exposing them to disease-free *Ixodes scapularis* ticks. Before tick exposure, we took a fecal sample of the mice, and we waited one week for all engorged ticks to fall off the mice. We then took another fecal sample. After this, we waited one week and repeated the process. In total, this gives us two sets of before and after samples for each mouse. Not only will this study give us clearer results on the effects tick parasitism has on mouse microbiomes, but it will help us see if the mouse microbiome composition rebounds after tick parasitism and to what extent it rebounds.

Our experimental studies currently focus on how tick parasitism can affect microbiome composition. In future experiments, we can shift our focus towards how tick-borne pathogens affect rodent gut microbiomes. Using a similar experimental set-up to study #2 and our ongoing study, we can expose mice to infected ticks, uninfected ticks, and no ticks and observe the shifts in gut microbiome composition. Additionally, we can expose the mice to tick-borne pathogens directly without using ticks as vectors to elucidate how tick-borne pathogens affect the mouse gut microbiome without having to take tick parasitism effects into account. We can also perform microbiome analysis on skin samples of the tick-parasitized vs unparasitized mice to better understand how tick parasitism affects rodent skin microbiomes.

Conclusion

High-throughput parallel sequencing has made way for novel microbiome studies. We now know that the gut microbiome composition can affect overall organism health and behavior. Additionally, we know that the gut and skin are linked via a gut-skin axis and that pathogens can affect the gut microbiome. We wanted to know how vector parasitism and vector-borne pathogens affect the host gut microbiome. Studies have been done on how the vector microbiome affects pathogen infection and transmission, but fewer studies have been done on how vector parasitism and pathogens affect the microbiomes of hosts. We were specifically interested in how ticks and tick-borne pathogens affect the gut microbiomes of rodent hosts. We conducted two studies: a sampling study of rodents in central Virginia and an experimental tick exposure study. From our first study, we determined that *B. burgdorferi* may potentially have an affect on microbiome richness in *P. leucopus* mice and we showed that gut microbiome richness and diversity were lower in *S. hispidus*. The taxon Muribaculaceae was in significantly higher abundance in parasitized mice, and this taxon is involved in carbohydrate metabolism, and it can potentially compete with harmful microbes such as *C. difficile*. This same taxon was shown to increase in abundance in the experimental study as a result of parasitism. Since this taxon increases in response to parasitism in two different rodent systems, it is plausible that this phenomenon is a common response to stress amongst rodent species. The experimental study also suggests that parasitism causes a change in rodent microbiome composition, and the origin of the tick involved in parasitism also can affect gut microbiome changes. We hope to conduct more studies relating tick parasitism to gut microbiome changes over more time steps in order to get a better sense of how rodent gut microbiomes can rebound after changing due to a stressor.

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Supplemental Tables and Figures

Table S1. Individuals sampled at each of three field sites in Virginia, USA, as well as tick parasitism information and infection status with B. burgdorferi. Approximate site coordinates are as follows: Goochland 37.58N -77.67W, Fort Pickett 37.08N -77.92W, Chesapeake 36.67N -76.36W.

Sampling location	Rodent species	Individuals sampled	Individuals with ticks	Individuals infected with *Borrelia burgdorferi*
Goochland	*P. leucopus*	23	9	3
	S. hispidus	3	0	0
Fort Pickett	*P. leucopus*	17	6	4
	S. hispidus	7	3	1
Richmond	*R. norvegicus*	11	0	NA
Chesapeake	*P. leucopus*	2	0	1

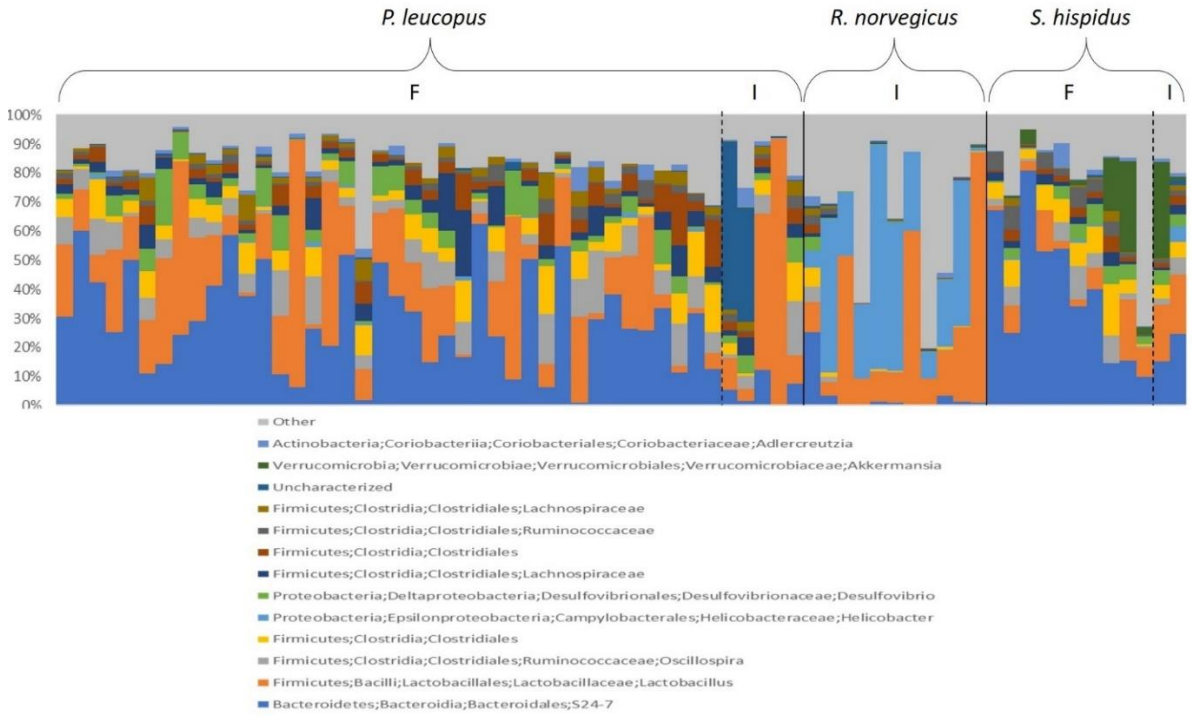

Figure 1S: *Relative abundance of the 13 most abundant bacterial taxa detected in this study, arranged by host species and sample type (F = fecal, I = intestine).*