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Interactions between Parasitic Infections and the Human Gut Microbiome in Odisha, India

Tiffany Huwe,[1] Birendra Kumar Prusty,[2] Aisurya Ray,[3] Shaun Lee,[1] Balachandran Ravindran,[3] and Edwin Michael[1]*
[1]*Department of Biological Sciences, University of Notre Dame, Notre Dame, Indiana;* [2]*District Diagnostic Laboratory, Malkangiri, India;* [3]*Institute of Life Sciences, Bhubaneswar, India*

Abstract. Soil-transmitted helminth (STH) infections and malaria are parasitic diseases with enormous global health burdens. Research has demonstrated a relationship between each of these parasites and the gut microbiome, suggesting that the gut microbiota may be implicated in governing host susceptibility to diverse pathogens, and perhaps even coinfection by different pathogens, through similar microbiome-influenced pathways. Here, we have derived a first microbiome community profile associated with STH infections in Odisha, India, and tested the hypothesis that the gut microbiome can modulate host susceptibility to multiple parasite infections through the same pathways. This study revealed several bacterial taxa negatively associated with specific STH infections, including *Lactobacillus* and Lachnospiracaea. Our results also suggest that relative abundance of *Lactobacillus* is driven by the STH infection status more so than by the *Plasmodium* infection status. This study contributes to efforts to understand the effects of the microbiome on host susceptibility to parasitic infections in endemic communities.

The gut microbiome consists of trillions of bacteria, which exert powerful effects on host nutrition, development, and immunity.[1,2] Dysbiotic microbiomes, often with reduced diversity, have been implicated in numerous chronic inflammatory conditions, including obesity, allergies, irritable bowel syndrome, and type I diabetes.[3,4] Gut microbiomes are also known to modulate host susceptibility to infectious pathogens, particularly intestinal parasites, via effects on both local and systemic immune responses.[5–7] Recent human field studies, for example, have reported increased diversity among microbiomes of helminth-infected subjects, suggesting a protective role against inflammatory disease in the host.[7–10] Helminths that reside in the gut can directly affect the immune system and are thought to benefit indirectly from mutualistic relationships with enteric microbes that can influence immune responses at the intestinal mucosa.[10,11] Although less intuitive, there is evidence that gut microbiota can also associate with *Plasmodium* parasites, perhaps through interactions mediated by the immune system.[12] A mouse study reported a negative association of *Bifidobacterium* and *Lactobacillus* with severe malaria, and a human field study reported a negative association of *Bifidobacterium*, *Streptococcus*, and Enterobacteriaceae with *Plasmodium falciparum* infection.[13,14] Another study has indicated that gut helminths may also be associated with enteric *Lactobacilli* species,[6] suggesting the intriguing possibility that the gut microbiota may be implicated in governing host susceptibility to diverse pathogens, and perhaps even coinfection by different pathogens, through similar microbiome-influenced pathways.

A pilot study was conducted in Odisha, India, where both soil-transmitted helminth (STH) infections and malaria are endemic.[15,16] The study was approved by the Institutional Review Boards at the University of Notre Dame and the Institute of Life Sciences, Bhubaneswar. Informed consent was obtained from all adult subjects and from a guardian of all minor subjects. The study was conducted in two phases; the first aimed to provide an initial profile of the association between microbiota and STH infections in this region. The second phase specifically examined the association between *Lactobacillus* and parasite coinfection. For the STH phase, stool samples were taken from subjects aged 2–5 years recruited from urban slums in Bhubaneswar, Odisha ($n = 16$). Stool collection kits were distributed to parents of potential subjects and collected 24 hours later. Common STHs *Ascaris lumbricoides*, *Necator americanus*, and *Trichuris trichiura* were detected through microscopy, flotation, and polymerase chain reaction (PCR) (for *N. americanus* and *T. trichiura* only). DNA was extracted from stool samples, and bacterial 16S rRNA was sequenced using the Illumina platform (Illumina Inc., San Diego, CA). Sequences were dereplicated, denoised, and clustered de novo into operational taxonomic units (OTUs) using UPARSE.[17] Data were analyzed using R version 3.4.3 (R Core Team, Vienna, Austria) and the Linear discriminant analysis Effect Size online interface.[18]

For the *Plasmodium* phase, blood and stool samples were taken from subjects of all ages recruited from rural villages near Rourkela, Odisha ($n = 68$). Stool collection kits were distributed and collected the same day. DNA extracted from blood and stool samples was used for PCR detection of *P. falciparum*, *Plasmodium vivax*, *N. americanus*, and *T. trichiura*. Fecal DNA from each sample in the *Plasmodium* phase was used as the template in one qPCR using Universal Bacteria primers and one quantitative PCR (qPCR) using *Lactobacillus*-specific primers. Cycle threshold (CT) values were recorded as measures of total bacteria and *Lactobacillus* abundance. The cycle threshold of Universal Bacteria was subtracted from the CT of *Lactobacillus* for each sample, yielding a value representing the inverse relative abundance of *Lactobacillus*.

One or more species of STH was detected in 87.5% of samples in the STH phase of the study. Each of the common STHs, *A. lumbricoides*, *N. americanus*, and *T. trichiura*, was present in this subset of samples. *Necator americanus* was the most prevalent STH (63%) followed by *A. lumbricoides* and *T. trichiura* (both 44%). Bacteria from 14 phyla were detected. The relative abundance of bacteria in each phylum varied among samples; however, Bacteroides, Firmicutes, and Proteobacteria consistently made up the largest portions of the microbiomes (Figure 1). The most abundant genus was *Prevotella* with a mean relative abundance of 40.3%.

Shannon diversity did not vary in *Ascaris*- ($t = 0.21$, $P = 0.84$, 2-tailed t-test), *Necator*- ($t = 0.31$, $P = 0.76$, 2-tailed t-test), and *Trichuris*-infected ($t = 0.137$, $P = 0.19$, 2-tailed t-test) individuals versus uninfected controls. Average Bray–Curtis beta diversity (between individual diversity) was greater among

* Address correspondence to Edwin Michael, University of Notre Dame, 349 Galvin Life Science Center, Notre Dame, IN 46556. E-mail: emichael@nd.edu

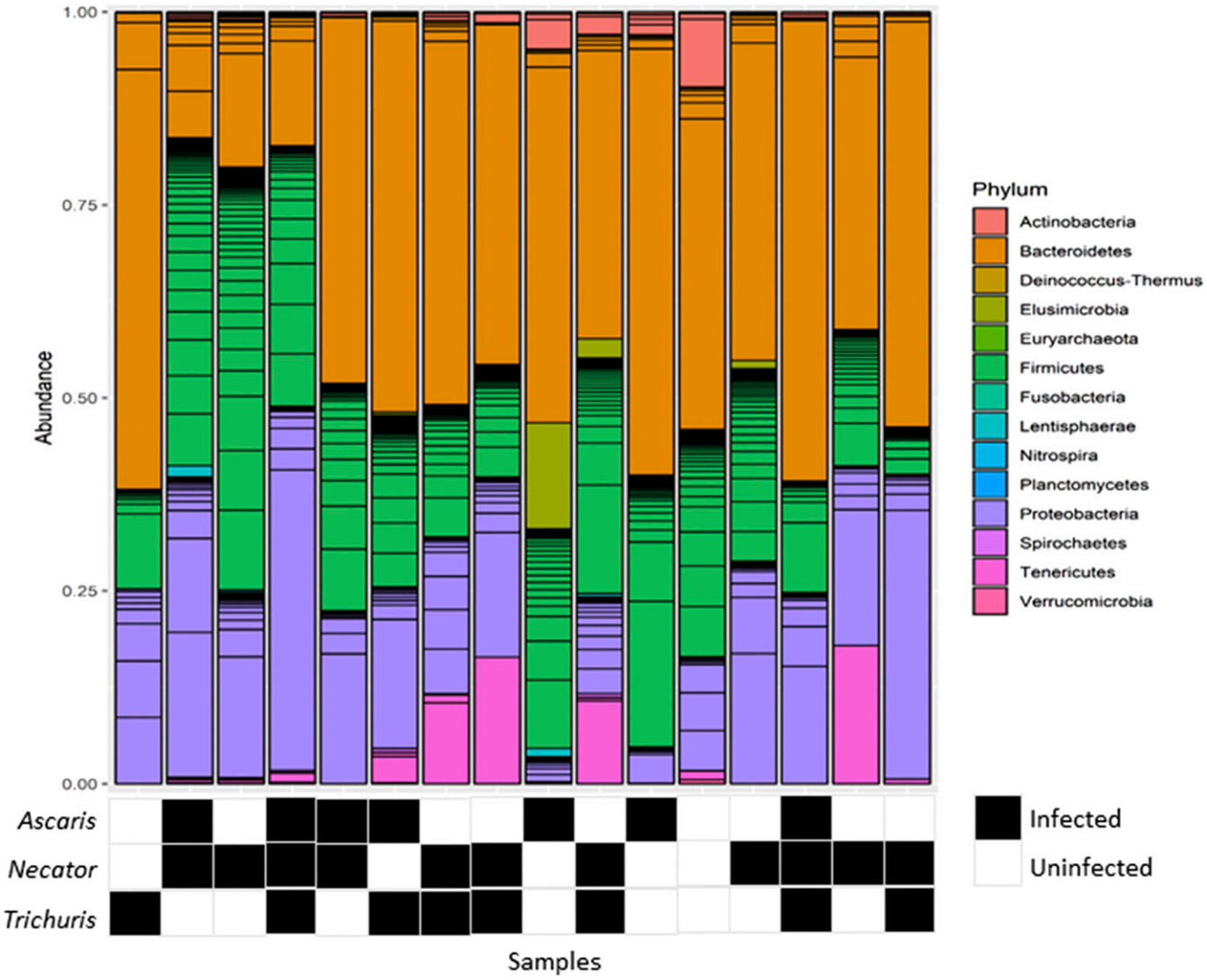

FIGURE 1. Relative abundance of phyla in the human gut microbiome. Lines within phyla show the contribution of individual operational taxonomic units. The relative abundance of phyla varied among samples.

Necator-infected (M = 0.54) than *Necator*-uninfected (M = 0.45) individuals (t = 2.21, P = 0.03, 2-tailed t-test). Approximately 13% of variation in Bray–Curtis dissimilarity could be explained by the *Necator* infection status (R^2 = 0.13). However, average Bray–Curtis beta diversity did not vary significantly in STH-, *Ascaris*-, and *Trichuris*-infected individuals versus controls.

Linear discriminant analysis Effect Size analysis (α = 0.05) identified several taxa as bacterial biomarkers of STH infection status. The mean relative abundance of *Lachnospiracaea_OTU_2* was reduced among *Ascaris*-infected (n = 7) versus *Ascaris*-uninfected samples (n = 9). The mean relative abundance of *Lactobacillus_OTU_2*, *Lachcnospiracaea_OTU_3*, and *Lachnospiracaea_OTU_4* was reduced among *Necator*-infected (n = 10) versus *Necator*-uninfected samples (n = 6). The mean relative abundance of *Lachnospiracaea_OTU_1*, *Dorea*, *Bifidobacterium*, and *Olsenella* was reduced among *Trichuris*-infected (n = 7) versus *Trichuris*-uninfected samples (n = 9).

Of the 68 subjects sampled in the *Plasmodium* phase of the study, 43% were female (Tables 1 and 2). According to the WHO BMI and BMI-for-age classification guidelines, 23% of subjects were underweight and 13% of subjects were overweight or obese. The STHs *N. americanus* and *T. trichiura* were detected in 8.5% and 3.4% of samples, respectively. Using rapid diagnostic tests, *Plasmodia* were detected in 46% of blood samples. Using the PCR diagnostic method, *P. vivax* was detected in 18% of blood DNA samples and *P. falciparum* was detected in 15% of blood DNA samples. Both *P. vivax* and *P. falciparum* were more prevalent among younger subjects than older subjects.

Stepwise logistic regression analysis indicated that education, number of children, and *Lactobacillus* were the best predictors of the *Plasmodium* infection status in our sample (akaike information criterion [AIC] = 36.29). All three variables were associated with reduced odds of infection; however, none were significant predictors at α = 0.05. The true predictors of *Plasmodium* infection were likely too numerous and complex for our limited survey to detect.

Analysis of the uninfected, helminth-only, *Plasmodium*-only, and coinfection status revealed that the median relative abundance of *Lactobacillus* was highest in microbiome

TABLE 1
Characteristics of Plasmodium study subjects by age group

Characteristic	Age group						All (*n* = 68)
	0–17 (*n* = 9)	18–25 (*n* = 15)	26–35 (*n* = 15)	36–45 (*n* = 15)	46–55 (*n* = 9)	56–60 (*n* = 5)	
Female	2 (22%)	7 (47%)	6 (40%)	6 (40%)	6 (67%)	2 (40%)	29 (43%)
RDT positive for *Plasmodium*	6 (67%)	8 (53%)	9 (60%)	6 (40%)	0	2 (40%)	31 (46%)
PCR positive for *Plasmodium*							
Plasmodium vivax	4 (44%)	4 (27%)	3 (20%)	1 (7.7%)	0	0	12 (18%)
Plasmodium falciparum	4 (44%)	3 (20%)	3 (20%)	0	0	0	10 (15%)
PCR positive for soil-transmitted helminths (number positive/number tested)							
Necator americanus	0/7	0/13	1/14 (7.1%)	0/13	0/7	1/5 (20%)	2/59 (3.4%)
Trichuris trichiura	0/7	3/13 (23%)	1/14 (7.1%)	0/13	0/7	1/5 (20%)	5/59 (8.5%)
Nutritional status by BMI (number/number tested)							
Underweight	3/8 (38%)	2/12 (17%)	4/12 (33%)	2/14 (14%)	1/9 (11%)	2/5 (40%)	14/60 (23%)
Overweight	1/8 (13%)	0/12	3/12 (25%)	1/14 (7.1%)	1/9 (11%)	1/5 (20%)	7/60 (13%)

samples from *Plasmodium*-only subjects (Figure 2). An analysis of variance (ANOVA) test confirmed that the mean relative abundance of *Lactobacillus* differed significantly in at least one group ($F(3,96) = 18.96$, $P < 0.001$). A Tukey test revealed significant differences in the relative abundance of *Lactobacillus* between the *Plasmodium*-only and each other group (*Plasmodium*-none *p-adj* < 0.001, *Plasmodium*-helminth *p-adj* < 0.001, *Plasmodium*-both *p-adj* = 0.02). The test did not detect a significant difference between the helminth-only group and those with coinfection or no infection (Helminth-both *p-adj* = 0.85; Helminth-none *p-adj* = 0.83).

The gut microbiomes described in this study of Bhubaneswar children match the profile of gut microbiomes previously reported in children living in rural India and Burkina Faso. All three studies found that gut microbiomes were dominated by the phyla Proteobacteria, Firmicutes, Bacteroidetes, and the genus *Prevotella*.[19,20] *Prevotella* have been functionally linked to diets high in plant fiber, as they secrete enzymes that help metabolize cellulose into short-chain fatty acids known to protect against gut inflammation.[20]

This study also identified numerous microbiome characteristics associated with STH infections. Beta diversity was generally higher in STH-infected subjects, although a significance difference was observed only for *Necator* infections, in agreement with the findings of Jenkins, Lee, Kay, and Rosa.[7–10] The direction of this relationship and the mechanism of action are not yet understood; however, the association between STH infection and increased microbiome beta diversity has been observed repeatedly. Perhaps helminth invasion of the intestine disturbs its ecological balance, causing once-similar microbiomes to diverge as they adjust.[21] Additionally, we also found greater relative abundance of bacteria belonging to *Lactobacillus*, Lachnospiracea, and *Olsenella* taxa in uninfected samples than in infected samples. Our findings regarding Lachnospiracea are consistent with those reported by Rosa et al.,[10] but our findings regarding *Olsenella* show disagreement. This disagreement may be the result of geographic variability in the microbiome behavior or a type II error due to the small sample size of this study. The STH phase was further limited by the low resolution of 16S sequence data, which cannot identify species or genes.

As sample collection was conducted in the dry season associated with low malaria transmission, the proportion of subjects infected with *Plasmodium* was predictably low. Because of reports that *P. falciparum* was the more prevalent *Plasmodium* species in Odisha, we were surprised to observe higher rates of *P. vivax* in this sample.[16] There were also high rates of coinfection. Each subject infected with *P. falciparum*

TABLE 2
Multivariate logistic regression analysis of risk factors associated with *Plasmodium* infection in rural villages in Odisha, India

Variable	COR	95% CI	*P*-value
Education			
Less than primary school	Ref	–	–
Completed primary school	0.21	0.01–1.73	0.194
Number of children	0.51	0.18–0.98	0.098
Lactobacillus measure CT (Lacto)-CT (Universal)	0.89	0.71–1.09	0.279

$F(45,48) = -8.15$, $P = 0.043 < 0.05$, Nagelkerke $R^2 = 0.29$, AIC = 36.29. COR = crude odds ratio.

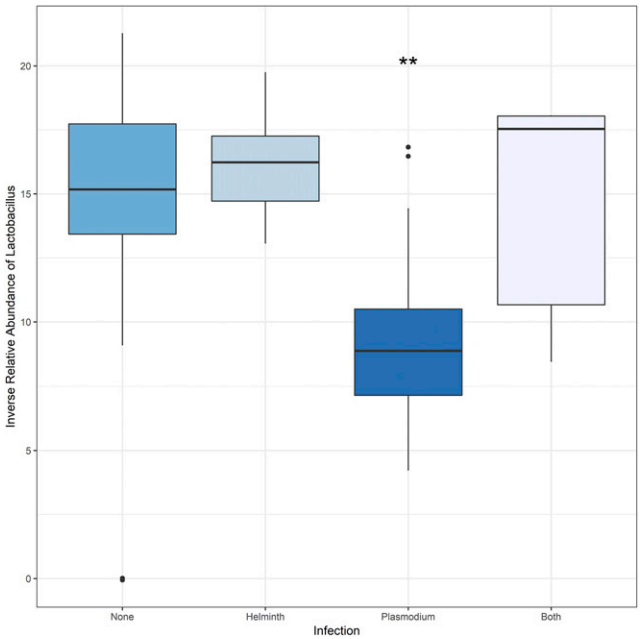

FIGURE 2. Relative abundance of *Lactobacillus* in gut microbiome samples by the *Plasmodium* and soil-transmitted helminth infection status. Distributions of CT (*Lactobacillus*)—CT (Universal Bacteria) values are shown for each infection status: uninfected, helminth-only, *Plasmodium*-only, helminth/*Plasmodium* coinfection. The relative abundance of *Lactobacillus* was significantly greater for *Plasmodium* infection–only samples than any other group (*Plasmodium*-None: $P < 0.001$; *Plasmodium*-helminth: $P < 0.001$; *Plasmodium*-both: $P < 0.02$). None, helminth, and both groups displayed statistically similar relative abundances of *Lactobacillus*. This figure appears in color at www.ajtmh.org.

also carried *P. vivax*. Analysis of coinfections revealed that *Lactobacillus* abundance was similar in uninfected, helminth-only, and *Plasmodium*/helminth coinfected microbiomes. With greater sample size, we might have distinguished the uninfected controls from the other groups. Our sample size was sufficient to determine that *Lactobacillus* was significantly more abundant in *Plasmodium*-only microbiomes. This suggests that *Lactobacillus* abundance observed in coinfected microbiomes is driven by helminths more so than *Plasmodium*, possibly due to the direct contact between helminths and microbiota in the GI tract. However, the association found with the two social factors related to educational status and number of children in a family investigated in this study point to the existence of more complicated pathways which may govern this relationship (Table 2). The results of this study are based on observational data and must not be used to draw conclusions about cause and effect. Further, because of convenient sampling, our small sample may have included only a narrow swath of the Rourkela and Bhubaneswar populations.

This study contributes to efforts to understand effects of the microbiome on host susceptibility to parasitic infections in endemic communities. We have derived a first microbiome community profile associated with STH infections in Odisha and tested the hypothesis that the gut microbiome can modulate host susceptibility to multiple parasite infections through the same pathways. We observed increased *Lactobacillus* among *Plasmodium*-only subjects but reduced *Lactobacillus* among STH-only subjects and those carrying coinfections (Figure 2). This indicates that relative abundance of *Lactobacillus* may influence susceptibility to either type of parasite, but in opposite directions. It may also govern coinfection with STH infections and malaria in this setting. A larger study is required to confirm these first findings and to identify the mechanisms behind the observed associations between the gut microbiome and host infection.

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Authors' addresses: Tiffany Huwe, Shaun Lee, and Edwin Michael, University of Notre Dame, Notre Dame, IN, E-mails: thuwe@nd.edu, shaun.w.lee.310@nd.edu, and emichael@nd.edu. Birendra Kumar Prusty, District Diagnostic Laboratory, Malkangiri, India, E-mail: drbirendraprusty@gmail.com. Aisurya Ray, Institute of Life Sciences, Bhubaneswar, India, E-mail: ray.aisurya123@gmail.com. Balachandran Ravindran, Institute of Life Sciences, Bhubaneswar, India, E-mail: ravindran8@gmail.com.

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