

# Natural Infection with *Giardia* Is Associated with Altered Community Structure of the Human and Canine Gut Microbiome

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**ABSTRACT** Enteric parasitic infections are among the most prevalent infections in lower- and middle-income countries (LMICs) and have a profound impact on global public health. While the microbiome is increasingly recognized as a key determinant of gut health and human development, the impact of naturally acquired parasite infections on microbial community structure in the gut, and the extent to which parasite-induced changes in the microbiome may contribute to gastrointestinal symptoms, is poorly understood. Enteric parasites are routinely identified in companion animals in the United States, presenting a unique opportunity to leverage this animal model to investigate the impact of naturally acquired parasite infections on the microbiome. Clinical, parasitological, and microbiome profiling of a cohort of 258 dogs revealed a significant correlation between parasite infection and composition of the bacterial community in the gut. Relative to other enteric parasites, *Giardia* was associated with a more pronounced perturbation of the microbiome. To compare our findings to large-scale epidemiological studies of enteric diseases in humans, a database mining approach was employed to integrate clinical and microbiome data. Substantial and consistent alterations to microbiome structure were observed in *Giardia*-infected children. Importantly, infection was associated with a reduction in the relative abundance of potential pathobionts, including *Gammaproteobacteria*, and an increase in *Prevotella*—a profile often associated with gut health. Taken together, these data show that widespread *Giardia* infection in young animals and humans is associated with significant remodeling of the gut microbiome and provide a possible explanation for the high prevalence of asymptomatic *Giardia* infections observed across host species.

**IMPORTANCE** While enteric parasitic infections are among the most important infections in lower- and middle-income countries, their impact on gut microbiota is poorly understood. We reasoned that clinical symptoms associated with these infections may be influenced by alterations of the microbiome that occur during infection. To explore this notion, we took a two-pronged approach. First, we studied a cohort of dogs naturally infected with various enteric parasites and found a strong association between parasite infection and altered gut microbiota composition. *Giardia*, one of the most prevalent parasite infections globally, had a particularly large impact on the microbiome. Second, we took a database-driven strategy to integrate microbiome data with clinical data from large human field studies and found that *Giardia* infection is also associated with marked alteration of the gut microbiome of children, suggesting a possible explanation for why *Giardia* has been reported to be associated with protection from moderate to severe diarrhea.

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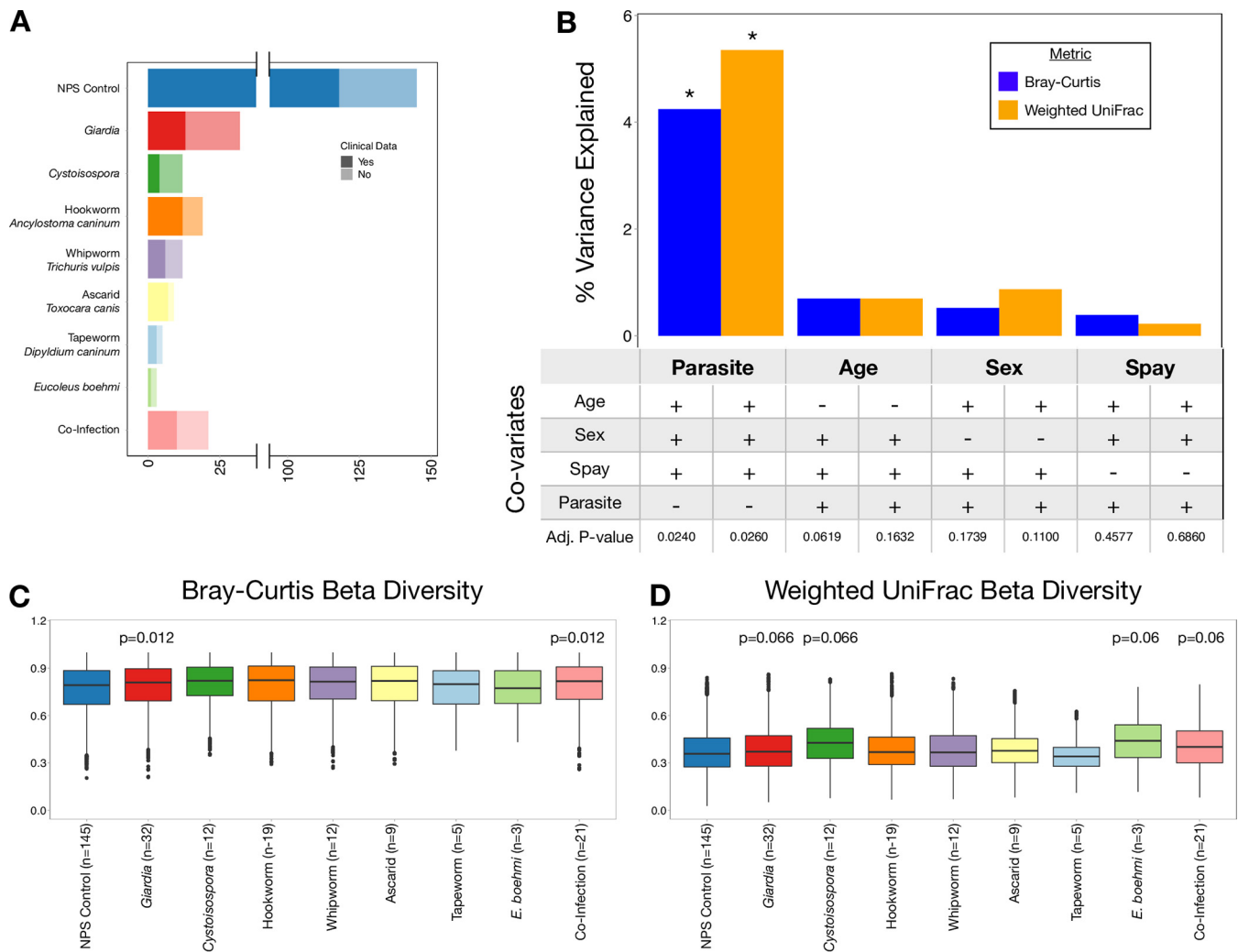
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Enteric parasites, including helminths and protozoa, are among the most prevalent infections in lower- and middle-income countries (LMICs) with an estimated 3.5 billion people affected worldwide (1, 2). Infection with eukaryotic pathogens often results in acute, moderate to severe diarrheal disease and/or chronic malnutrition and stunting, which has significant consequences for morbidity and mortality (3–5). Conversely, some intestinal parasites are frequently associated with asymptomatic infections (6, 7). *Giardia*, for example, was found in 18 of 1,093 (1.6%) of healthy volunteers in Melbourne, Australia (8) and in 286 of 1,359 (21%) of healthy schoolchildren in Madrid, Spain (9). It is important to understand whether and how these abundant and pervasive parasites impact gut health.

While the microbiome is increasingly recognized as a key determinant of gut health and human development, the impact of naturally acquired parasite infections on the microbial community in the gut is poorly understood. Many studies of parasites and their impact on the microbiome involve experimental infections of laboratory animals (10–15). While such studies can be powerful for elucidating mechanisms, they often involve laboratory-adapted parasite strains, specialized animal husbandry practices, or high infectious doses, all of which can impact host immunity and the composition of the microbiome. Conversely, studies of parasite infections in human populations are challenging due to the relatively low prevalence of these infections in developed countries and the presence of confounding variables in LMICs, such as malnourishment and coinfections (16–19). These issues are largely overcome by studying enteric parasite infections in companion animals. Various enteric parasites are frequently found in screenings of domestic dogs and cats in the United States (20). For example, a study of over one million dogs throughout the United States in 2006 found that 12.5% were infected with at least one enteric parasite, with the most prevalent being *Giardia* which infected 4% of dogs (21). As companion animals, dogs are increasingly recognized as an ideal model system for translational gut microbiome research. In addition to harboring similar gut microbiota as humans, dogs often share their environment with humans, consume a similar omnivorous diet, and can spontaneously develop gastrointestinal (GI) disease that shares many features in common with inflammatory bowel disease in humans (22–29). In addition, like humans, dogs frequently become infected with enteric parasites in early life. Here, we performed 16S rRNA sequencing of fecal samples from 258 dogs naturally infected with one or more eukaryotic parasites to evaluate the impact of parasite infection on gut microbiota composition. We found that parasite infections are associated with significant perturbations to the microbiome and that *Giardia* is associated with the largest changes in canine gut microbiota.

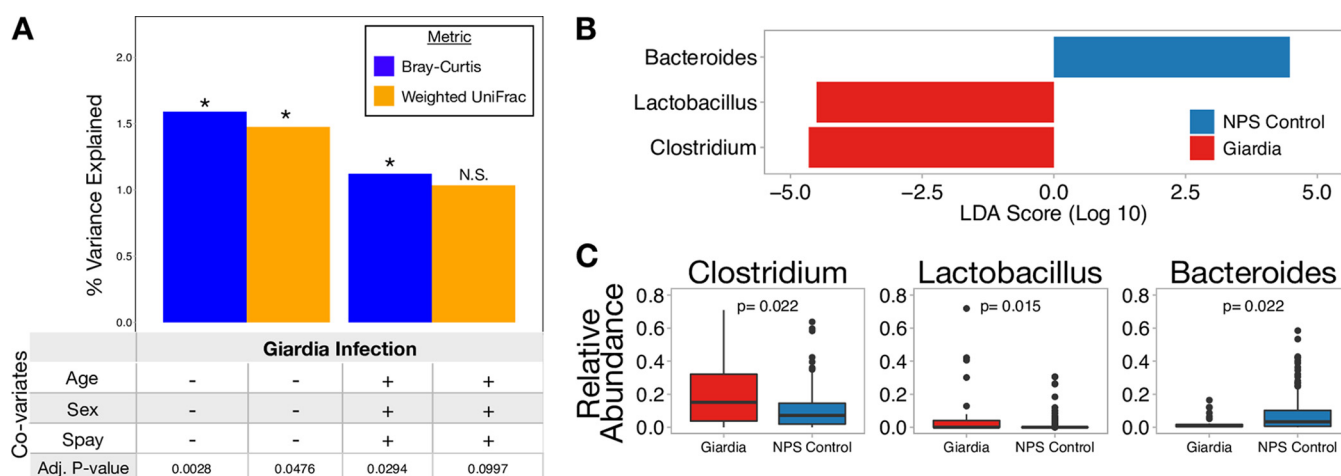
We also investigated whether *Giardia*—a frequent infection among humans residing in LMICs—causes similar perturbations in human gut microbiota composition. The Global Enteric Multicenter Study (GEMS) investigated the causes of pediatric moderate to severe diarrhea (MSD) in LMICs (30). In addition to reporting a strong association between infection with rotavirus or *Cryptosporidium* and the development of MSD, this study also reported the surprising observation that *Giardia* was found more often among asymptomatic participants than those with MSD in this cohort (30), despite the association between *Giardia* and serious chronic health conditions, including growth stunting (31), irritable bowel syndrome (IBS), and fatigue (32). A follow-up to the GEMS study performed 16S sequencing of fecal samples from approximately 1,000 GEMS participants (33), but this study only considered the relationship between the microbiome and MSD and did not examine a role for parasite infections in influencing this relationship. Similarly, The Etiology, Risk Factors, and Interactions of Enteric Infections and Malnutrition and the Consequences for Child Health (MAL-ED) study investigated the hypothesis that infection with enteric pathogens contributes to undernutrition in children (34). A follow-up to the MAL-ED study performed 16S sequencing on nearly 1,000 fecal samples from participants in the Peruvian cohort to assess the taxa asso-



ciated with the burden of *Campylobacter* and other enteric pathogens (35). We used a database mining approach to determine whether *Giardia* infection perturbs the human gut microbiome, in both the GEMS and MAL-ED cohorts, similarly to how it perturbs the canine gut microbiome, and to gain insight into possible mechanisms by which *Giardia* infection may be linked to protection against diarrhea in some individuals.

## RESULTS

**Enteric parasite infections perturb the canine microbiome.** A stool bank was generated from samples screened at a veterinary clinical parasitology service as part of our Companion Animal Microbiome during Parasitism (CAMP) study (see Materials and Methods). A total of 258 canine fecal samples were split into 9 groups based on parasite infection status (Fig. 1A): (i) no parasite seen (NPS) controls; (ii) *Giardia*, the causative agent of giardiasis; (iii) *Cystoisospora*, the causative agent of coccidiosis, an intestinal tract infection; (iv) hookworm, which causes intestinal distress and anemia; (v) whipworm, which causes severe irritation to the large intestine; (vi) ascarid, which causes weakness, diarrhea, and vomiting; (vii) tapeworm, which is generally asymptomatic but



**FIG 2** *Giardia* infection is associated with enrichment of several key bacterial taxa in the canine gut. (A) Histogram showing that *Giardia* infection is associated with a significant difference in beta diversity compared to NPS controls. Bar height reflects the percentage of total beta diversity variance that is explained by *Giardia* infection. Plus symbols below the bars show when age, sex, and spay/neuter status are controlled for. Asterisks denote bars with adjusted *P* values of  $<0.05$ . (B) LefSe graph shows the magnitude of enrichment with LDA score of  $>$  comparing *Giardia*-infected dogs to NPS control dogs. (C) Boxplots showing the relative abundance of differentially enriched taxa. *Clostridium* is among the most highly enriched bacterial taxa associated with *Giardia* infection compared to controls.

indicative of flea infestation and may cause perianal pruritis; (viii) *Eucoleus boehmi*, a parasite whose eggs are shed in the stool and that can cause chronic rhinitis; and (ix) dogs with coinfections of two or more of these parasites. Since certain enteric parasites, such as *Giardia*, are more prevalent in young animals, age and other potential confounding variables were controlled for in our statistical analyses. Parasite infection status was associated with significant changes in beta diversity, as determined by both Bray-Curtis and weighted UniFrac metrics, even when covariates such as age, sex, and spay/neuter status were controlled for as confounding variables (adjusted *P* value [Adj *P*]  $< 0.05$  by permutational multivariate analysis of variance [PERMANOVA]) (Fig. 1B). The significance of parasite infection remains unchanged when infections represented by fewer than 9 samples were removed from the analysis (tapeworm and *Eucoleus boehmi*). Approximately 5% of the variation in microbiome composition was explained by parasite infection status compared to  $<1\%$  explained by age, sex, or spay/neuter status alone (Adj *P*  $> 0.05$  by PERMANOVA) (Fig. 1B). Specifically, *Giardia*- and coinfecting animals displayed the most significant differences in beta diversity compared to NPS controls by both Bray-Curtis (Fig. 1C) and weighted UniFrac metrics (Fig. 1D).

**Canine *Giardia* infection is associated with significant alterations in gut microbiota composition.** Given the diverse range of parasites detected in our animals, we set out to determine whether specific types of parasites were associated with more pronounced microbiome alterations. *Giardia* infection is associated with a change in Bray-Curtis (Adj *P*  $< 0.01$ ; 1.6% of total variation) and weighted UniFrac (Adj *P*  $< 0.05$ ; 1.5% of total variation) beta diversity compared to NPS controls, without controlling for age, sex, and spay/neuter status (Fig. 2A). When controlling for age, sex, and spay/neuter status, beta diversity is still significantly altered during *Giardia* infection as measured by Bray-Curtis (Adj *P*  $< 0.05$ ; 1.1% of total variation), but no longer meets the 0.05 cutoff for significance for weighted UniFrac (Adj *P* = 0.0997; 1.0% of total variation) (Fig. 2A). The differences in beta diversity between *Giardia* infection and NPS controls were driven by several bacterial taxa as determined by linear discriminant analysis (LDA) effect size (LefSe) analysis (Fig. 2B and C). *Giardia* is associated with enrichment of *Clostridium*, a genus that contains several commensal taxa, as well as an enrichment of *Lactobacillus*. However, *Giardia* was also associated with a reduction in *Bacteroides*, a genus that includes important commensal bacteria. In order to verify the taxa associated with *Giardia* infection, point-biserial correlation coefficients were calculated for each taxon with average relative abundance of  $>1\%$ . Consistent with our LefSe

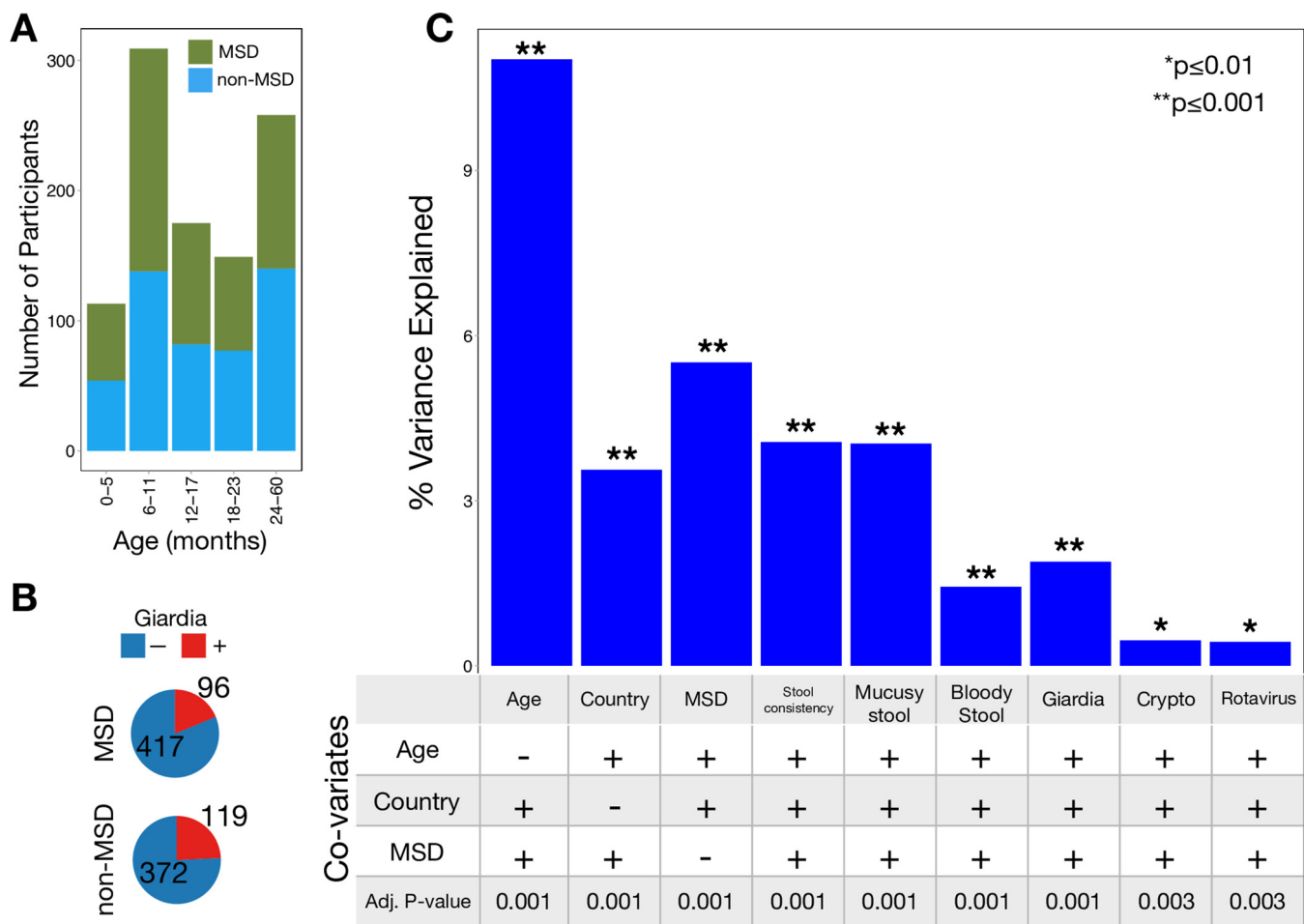
results, point-biserial correlation coefficients also showed enrichment of *Clostridium* and *Lactobacillus*, and a reduction in *Bacteroides* in addition to a reduction in *Megamonas* (see Table S1 in the supplemental material). The high relative abundance of *Clostridium* and *Lactobacillus* and the low relative abundance of *Bacteroides* in *Giardia*-infected dogs compared with NPS controls show that *Giardia* infection in animals is associated with an altered gut microbiota composition.

It is possible that the microbiota changes observed in *Giardia*-infected dogs could be driven, in part, by clinical variables such as diarrhea or antibiotic use. To discriminate between changes linked to diarrhea or antibiotics versus those linked to infection, we evaluated medical records, when available ( $n = 174$ ), to identify animals with a recent history of diarrhea or antibiotic use (Fig. 1A). Interestingly, *Giardia* infection was not associated with diarrhea or antibiotic use ( $P > 0.25$  by chi-squared test): among dogs with clinical data, 4 of 13 (30.1%) *Giardia*-infected dogs had diarrhea compared to 21 of 118 (18%) NPS control dogs with diarrhea (see Fig. S1A in the supplemental material), while 4 of 13 (15.4%) *Giardia*-infected dogs received antibiotics compared to 15 of 118 (12.7%) NPS control dogs (Fig. S2A). In contrast, antibiotic use was strongly correlated with diarrhea ( $P < 0.01$ ; chi-squared test), with most dogs on antibiotics having diarrhea (11/15) and over half of dogs with diarrhea being on antibiotics (11/21). Among NPS control animals with clinical data available ( $n = 118$  dogs) (Fig. S1A), those with diarrhea had significantly different Bray-Curtis beta diversity (Adj  $P < 0.001$ ; 2.9% of total variation) and weighted UniFrac beta diversity (Adj  $P < 0.01$ ; 3.7% of total variation) compared to asymptomatic animals; and those receiving antibiotics had significantly different Bray-Curtis beta diversity (Adj  $P < 0.001$ , 3.5% of total variation) and weighted UniFrac beta diversity (Adj  $P < 0.01$ ; 3.9% of total variation) compared to those not receiving antibiotics, when controlling for age, sex, and spay/neuter status (Fig. S1B). Next, we used our NPS control group ( $n = 118$ ) to define a microbiome signature associated with diarrhea in the absence of observable parasites, allowing us to compare this signature with *Giardia*-infected animals. LEfSe analysis identified *Escherichia* as enriched in animals with diarrhea and in those receiving antibiotics, while *Bacteroides* and *Fusobacterium* were enriched in asymptomatic dogs (Fig. S1C and S1D) and those not receiving antibiotics (Fig. S2B). Taken together, these data define a microbiome profile associated with diarrhea and antibiotic use in NPS animals that is marked by enrichment of *Escherichia* and *Fusobacterium* and show that this signature is distinct from that observed during *Giardia* infection (Fig. 2B and C).

**The effect of *Giardia* on the microbiome persists during coinfection.** We reasoned that if *Giardia*—compared to other parasites observed in our samples—is driving changes in the microbiome, then we should observe a similar profile in animals harboring coinfections with *Giardia* and at least one other parasite. Ten out of 21 dogs harboring multiple parasites (“coinfection”) were infected with *Giardia* and one or more other parasites. These 10 *Giardia* coinfecting samples were indistinguishable from *Giardia* singly infected animals by Bray-Curtis (Adj  $P > 0.1$ ) and weighted UniFrac (Adj  $P > 0.1$ ) beta diversity (Fig. S3). In contrast, *Giardia* singly infected samples were significantly different from the remaining 11 coinfecting samples not involving *Giardia* by Bray-Curtis (Adj  $P < 0.05$ ) and weighted UniFrac (Adj  $P < 0.05$ ) beta diversity; however, false discovery rate correction raises these  $P$  values slightly above the 0.05 significance threshold (Fig. S3). Taken together, these results show that *Giardia* infection in dogs is associated with a unique and significant change in gut microbiota composition compared to NPS controls that persist even in the context of coinfection with other parasites.

***Giardia* infection is among the largest predictors of the pediatric gut microbiota structure in the GEMS case-control study.** After finding that parasites, in particular *Giardia*, perturb the canine gut microbiome, we asked whether *Giardia* similarly affected the human gut microbiome. To this end, we employed a database mining approach to integrate and query data from the Global Enteric Multicenter Study (GEMS) and the Etiology, Risk Factors, and Interactions of Enteric Infections and Malnutrition

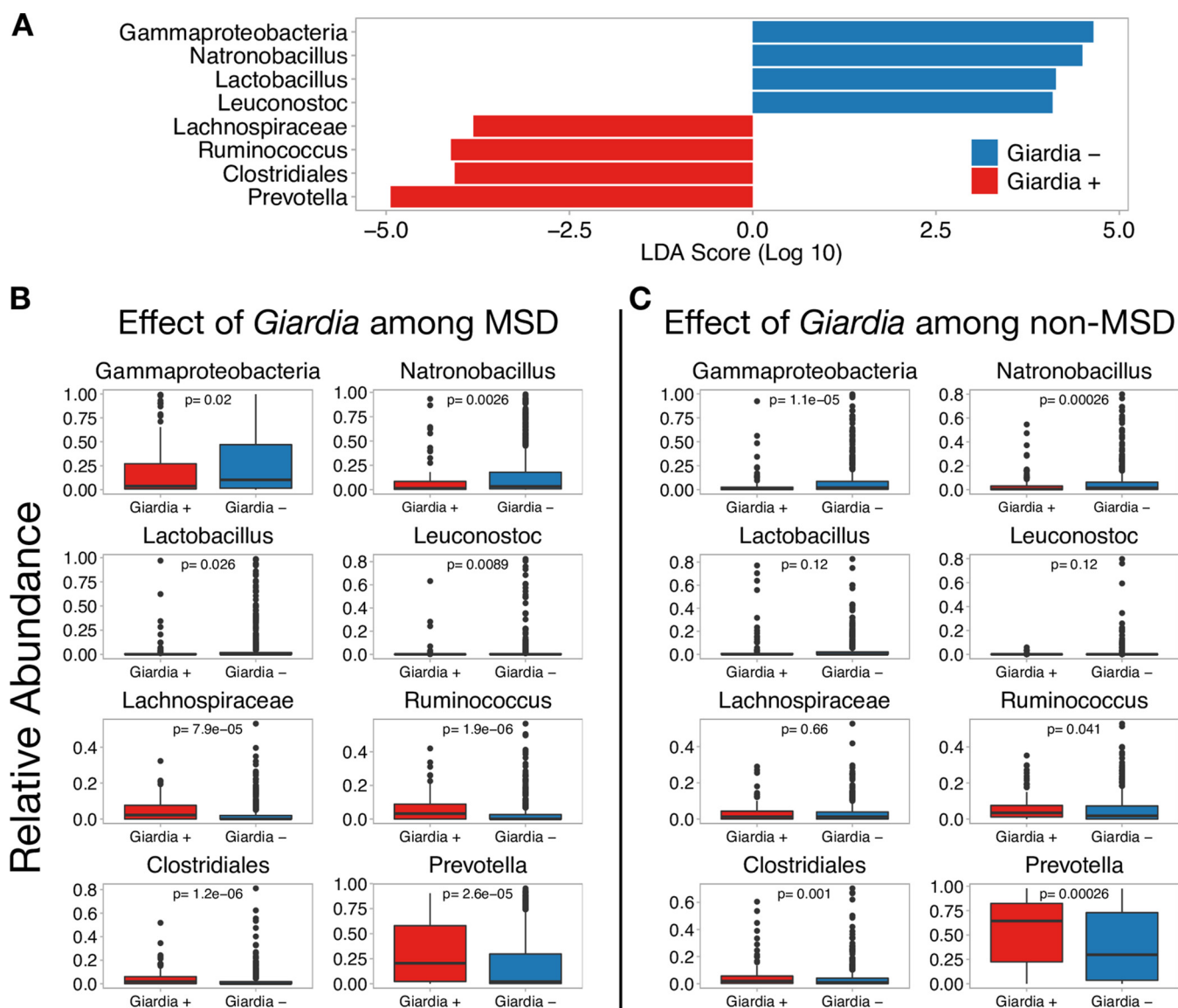




**FIG 3** Enteric parasites are associated with gut microbiota perturbations in children. (A) The number of participants with moderate to severe diarrhea (MSD) (green) and without MSD (blue) in each of five age cohorts is shown. (B) *Giardia* is more frequently found in children without MSD compared to children with MSD. (C) The percent variation in Bray-Curtis beta diversity explained by several variables is shown by bars. Whether the analysis was stratified by age, country, and/or MSD status is shown below each bar. *Giardia* is significantly associated with a change in gut microbiota and explains more microbiota variation than any other enteric pathogen detected here.

and the Consequences for Child Health (MAL-ED) study. Clinical and epidemiological data from GEMS were made available on ClinEpiDB.org (Fig. 3A and B) from over 22,000 participants. Previously published microbiome data from a subset of the same participants ( $n = 1,004$ ) were loaded on MicrobiomeDB.org (36, 37). Clinical and microbiome data were manually integrated, leading to the identification of 215 participants who were positive for *Giardia* and for which microbiome data were available. Not surprisingly, age and moderate to severe diarrhea (MSD) were strongly correlated with Bray-Curtis beta diversity (Adj  $P < 0.001$ ), explaining 11% and 5.5% of the total variation in microbiome structure, respectively (Fig. 3C). *Giardia* infection was associated with a similarly large perturbation of the gut microbiota (Adj  $P < 0.001$ ; 1.9% of the total variation), while *Cryptosporidium* and rotavirus infection were each associated with  $<0.5\%$  of the variation in microbiota composition in this cohort (Adj  $P < 0.01$ ). Only 14 of the 215 *Giardia*-infected children were also coinfecting with either *Cryptosporidium* or rotavirus. Infection with *Giardia* was also significantly associated with gut microbiota composition in each of the four countries individually (Fig. S4A).

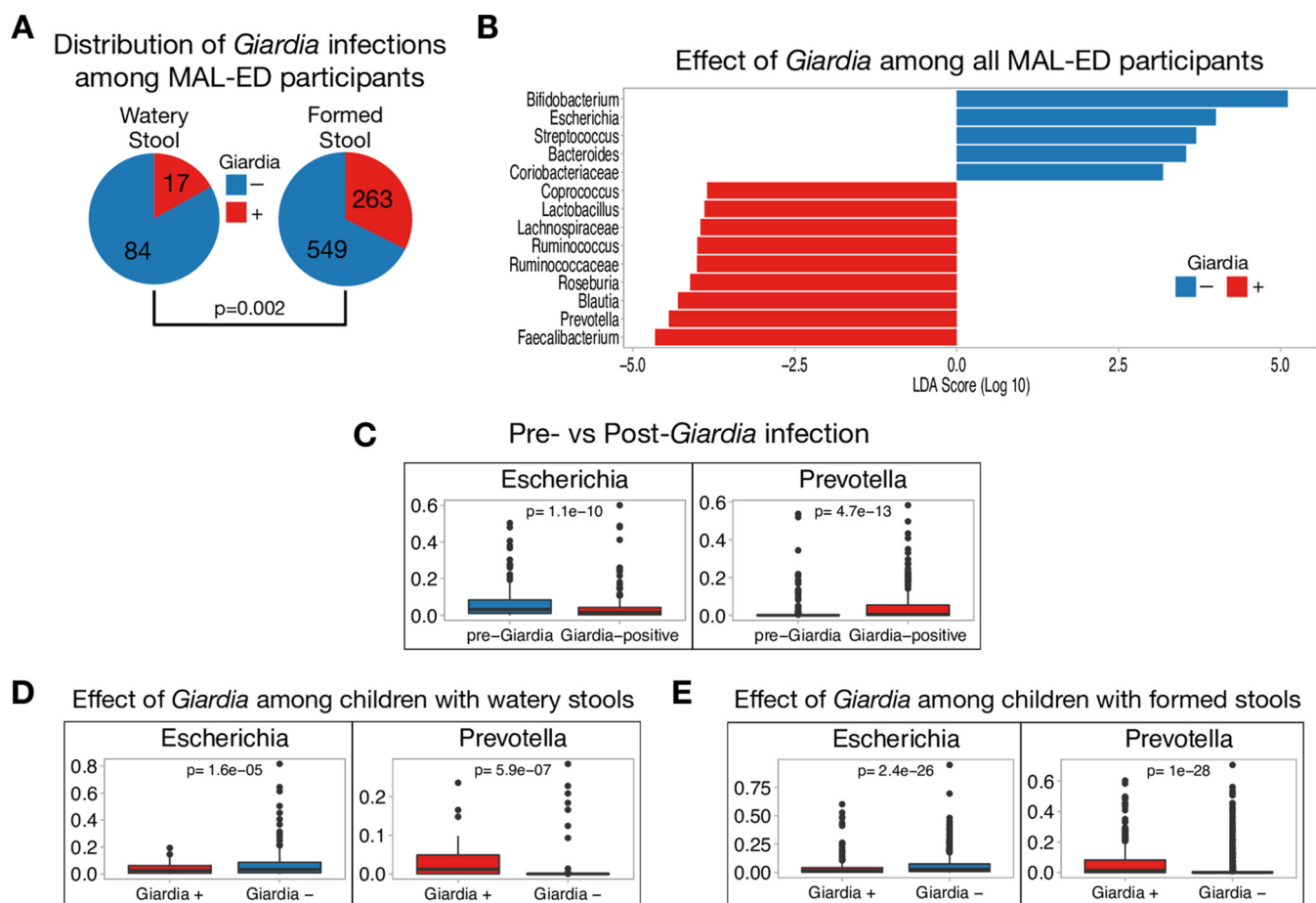
We observed that *Giardia* infection among GEMS participants was associated with enrichment of *Prevotella* and a reduction in *Gammaproteobacteria* (Fig. 4A)—an effect that was evident in children with MSD (Fig. 4B) and without MSD (Fig. 4C). LEfSe analyses performed on data partitioned by country showed that *Giardia* infection is associated with enrichment of *Prevotella* and a reduction in *Gammaproteobacteria* in all



**FIG 4** *Giardia* infection in children is associated with a reduction in *Gammaproteobacteria* regardless of disease status. (A) LefSe graph showing the magnitude of enrichment for each taxon with LDA score of  $>2$  comparing children with and without *Giardia* infection. (B and C) Boxplots showing the relative abundance of differentially enriched taxa among children with MSD (B) and those without MSD (C). A very similar set of taxa are differentially expressed during *Giardia* infection regardless of clinical disease. Although the relative abundance of *Gammaproteobacteria* and *Prevotella* are different between MSD and non-MSD, *Giardia* infection is significantly associated with a reduction of *Gammaproteobacteria* and enrichment of *Prevotella* regardless of MSD status. Taxa were collapsed to the genus level when possible; however, *Gammaproteobacteria*, *Clostridiales*, and *Lachnospiraceae* were only able to be collapsed to the class, order, and family, respectively.

four countries (Fig. S4B). Diarrhea is commonly associated with a reduction in *Prevotella* and an increased abundance of *Gammaproteobacteria*. Moreover, age strongly influences the relative abundance of *Prevotella* and *Gammaproteobacteria* (Fig. S5A and S5B, respectively), as well as *Giardia* prevalence (Fig. S5C and S5D). To control for these factors, the impact of *Giardia* was assessed among 12- to 17-month-old GEMS participants, a cohort with high relative abundance of both *Prevotella* and *Gammaproteobacteria* and high prevalence of *Giardia* infections (29.1%;  $n = 51$ ) and for which *Giardia* prevalence is not correlated with age ( $P = 0.99$  by chi-squared test). Among 12- to 17-month-old children, the association between *Giardia* infection and reduction in *Gammaproteobacteria* and enrichment of *Prevotella* remained (Fig. S6).

**Longitudinal tracking of *Giardia* status reveals infection-dependent alterations in microbiome composition.** We employed the same database mining strategy to



**FIG 5** *Giardia* is associated with a reduction in *Escherichia* and enrichment of *Prevotella* among Peruvian children in the MAL-ED study. (A) Among all Peruvian participants in the MAL-ED study, *Giardia* is more frequently found in children with formed or soft stools than in children with liquid or watery stools ( $P = 0.002$  by chi-squared test). (B) LefSe graph showing the magnitude of enrichment for all taxa meeting the adjusted  $P$  value threshold of 0.05, comparing children with (red) and without (blue) *Giardia* infection. Only taxa with relative abundance of  $>1\%$  across all samples were used in the analysis. (C) Boxplots showing the differences in relative abundance of *Escherichia* and *Prevotella* immediately before and after acquiring *Giardia* infection. (D) Boxplots showing that *Giardia* is associated with a reduction in *Escherichia* and enrichment of *Prevotella* among children with liquid or watery stools and (E) among children with formed or soft stools. All  $P$  values were adjusted using Benjamini-Hochberg multiple testing correction.

combine clinical and microbiome data from the MAL-ED study. Clinical and epidemiological data for 2,145 participants sampled longitudinally ( $>1.8$  million observations) were stored on ClinEpiDB.org and were manually integrated with fecal microbiome data (available on microbiomeDB.org) from 271 participants from the Peru cohort, each sampled up to four times at 6, 12, 18, and 24 months of life ( $n = 913$  samples) (34, 38). Of the 913 fecal samples, 280 were positive for *Giardia* by enzyme-linked immunosorbent assay (ELISA). *Giardia* was observed more frequently in formed or soft stool samples compared to liquid or watery stools ( $P = 0.002$  by chi-squared test) (Fig. 5A), consistent with the GEMS study, where *Giardia* infection was more prevalent among participants without MSD. *Giardia* infection among MAL-ED participants was associated with enrichment of *Prevotella* and a reduction in *Escherichia*, a member of the *Gammaproteobacteria* class (Fig. 5B). MAL-ED sequenced the V4 region of the 16S gene and was classified against the SILVA database, while GEMS sequenced the V1-V2 region and was classified against the Greengenes database, which likely led to some differences in taxonomic classification, including the identification of the *Escherichia* genus specifically in MAL-ED compared to the broader *Gammaproteobacteria* class in GEMS.

We reasoned that if *Giardia* infection was driving the change in microbiome composition observed in the GEMS data, then the longitudinal aspect of MAL-ED should allow identification of individuals that convert from *Giardia* negative to positive



during the study period, providing a unique context for examining parasite-microbiome interactions. A total of 170 pairs of MAL-ED observations were identified where a *Giardia*-positive stool sample could be compared to the *Giardia*-negative sample obtained from the same individual 6 months prior. Analysis of the microbiome in these discordant pairs showed that becoming infected with *Giardia* was marked by decreased relative abundance of *Escherichia* and increased *Prevotella* (Adj  $P < 1e-9$ ) (Fig. 5C). Since samples were collected 6 months apart, an effect of age cannot be ruled out. To address this, we also evaluated 53 pairs of observations where individuals converted from *Giardia* positive to negative over a 6-month period. In these cases, clearing *Giardia* was not associated with a change in relative abundance of *Escherichia* or *Prevotella* (Adj  $P > 0.1$ ), suggesting that the differences observed upon infection cannot be explained by age alone. These data also suggest that clearing *Giardia* does not have an inversely proportional effect on the gut microbiota composition as acquiring *Giardia*. Finally, the enrichment of *Prevotella* and reduction in *Escherichia* were evident in children with liquid or watery stools (Fig. 5D) as well as those with formed or soft stools (Fig. 5E). Taken together, these results demonstrate that *Giardia* infection leads to an altered gut microbiome structure in humans, marked by changes in the relative abundance of taxa linked to gut health (39–41).

## DISCUSSION

Enteric parasite infections are among the most common causes of diarrhea in humans in the developing world. While bacterial infections and the gut microbiome have been well-studied, the impact of enteric eukaryotic parasites on the microbiome is not well understood, with some reports showing altered microbiome composition (17, 42–46, 105) while others showed either modest or no impact (47–50). Because these studies often rely on experimental infection with one or few parasite species or observations using a small number of participants, they provide limited insight into the broader impact of enteric parasites on the gut microbiome. By combining clinical parasitology and microbiome profiling from humans and canines infected with a phylogenetically diverse range of enteric parasites, we show that naturally acquired enteric parasite infections are a major factor associated with microbiome composition, that this effect is observed across host species, and that *Giardia* is associated with the largest impact among all parasites surveyed in dogs and humans.

*Giardia* is one of the most common enteric parasites in the world and is remarkable in its ability to cause an array of clinical phenotypes, ranging from asymptomatic infection to severe acute diarrheal disease to chronic gastrointestinal disease. *Giardia* is the causative agent of giardiasis, a diarrheal illness, and is clearly implicated in serious growth stunting and long-term health consequences (51), cementing its role as a pathogen. However, our observations (Fig. 3), as well as other reports in humans and animals suggest that *Giardia* infection is frequently asymptomatic (8, 51–58). Intriguingly, several large epidemiological case-control studies recently showed higher *Giardia* prevalence in asymptomatic participants compared to those with moderate to severe diarrheal disease, revealing a possible protective role (30, 34, 51, 56, 59, 60). The negative association between *Giardia* and MSD may be due, in part, to asymptomatic participants shedding hardier cysts that preserve DNA better than the trophozoites typically associated with severe *Giardia* infection; however, data from this study and others suggest biological explanations for the phenomenon. *Giardia* infection may modulate symptoms in some individuals by modulating the immune response to other pathogens (56, 61), as seen in a recent study showing that *Giardia* coinfection attenuates the severity of disease caused by other enteric pathogens (62). We reasoned that parasite-induced perturbations in the microbiome could also be an important factor influencing gastrointestinal symptoms. Our results, in combination with similar results from recent smaller-scale mouse and human experiments (62, 63), raise the possibility that the shift in microbiome composition during *Giardia* infection—marked by a reduction in *Gammaproteobacteria* and an increase in *Prevotella*—may explain, at least

in part, the apparent protective effect of *Giardia* against diarrhea in some age/site cohorts (30, 34, 56).

One intriguing extension of our findings is the notion that *Giardia* may benefit directly from manipulation of the microbiome as seen during infection with the intestinal parasite *Blastocystis* which engulfs highly abundant bacterial taxa to meet its nutritional demands, causing drastic changes to gut microbiota composition (64). Infection by another protozoan parasite, *Entamoeba histolytica*, results in enrichment of *Escherichia coli* that protects the parasite from oxidative damage by producing malate dehydrogenase (65). Similarly, during infection with the helminth *Trichuris muris*, *Proteobacteria* directly interact with parasite eggs to induce hatching, thereby enhancing worm reproduction (66). Taken together, these studies highlight that eukaryotic parasites impact the microbiome in ways that can influence host health, immunity, and parasite biology.

Our data show an association between *Giardia* infection and microbiome composition but do not resolve whether infection is the primary driver of these changes. It is possible that certain microbiome compositions confer susceptibility or resistance to colonization by the parasite, as suggested by previous studies (67). Although it may seem surprising that a pathogen of the upper small intestine could have the potential to impact microbiome composition in the stool, recent studies of mice experimentally infected with *Giardia* revealed alterations of the gut microbiome throughout the small and large intestine, indicating both a causal role for infection in inducing these changes and the ability of the parasite to profoundly alter bacterial community structure far from the site of infection (12). Moreover, our analysis of longitudinal data from the MAL-ED study, which show a clear shift in composition commensurate with infection, argue in favor of microbiome changes being a consequence, rather than a cause, of infection.

Taken together, these data make a strong case for pursuing mechanistic studies that address interactions between *Giardia*, the microbiome, and the host. Interestingly, *Giardia* infection is associated with malabsorption of fats, leading to intestinal steatosis and increased transit of lipids into the distal small intestine and colon (68), malabsorption of sugars and proteins, and diffuse shortening of the intestinal brush border microvilli (69, 70). These pathological changes could alter substrate availability for commensal bacteria, providing a possible explanation for compositional changes in the microbiome during this infection. Additionally, gut microbiota composition changes could be caused by *Giardia*-induced disturbances to biofilm composition and structure which have been linked to dysbiosis (71, 72).

The age at which humans or animals are exposed to *Giardia* is thought to impact clinical manifestations. For example, there appears to be a window of time early in childhood development when *Giardia* infection is negatively associated with diarrhea. Studies of several pediatric cohorts show either no correlation or a negative correlation between *Giardia* infection and diarrhea (30, 51, 53–55, 73), although definitions of diarrhea vary from “loose stool” to clinically severe diarrhea. Notably, those studies that specify moderate to severe diarrhea show a significant association between *Giardia* infection and not having diarrhea (30, 54), suggesting that *Giardia* infection may be negatively associated with severe diarrhea but not loose stools. Indeed, clinical manifestations associated with *Giardia* infection are variable, likely due in part to variation among *Giardia* genotypes, and may explain why children in countries where *Giardia* is not endemic are more likely than children in countries where *Giardia* is endemic to have symptomatic *Giardia* infection (74–77). In contrast, adults—especially those in areas where *Giardia* is not endemic—show a positive correlation between *Giardia* infection and diarrhea (51, 78). Previous studies suggest that the association between growth stunting and *Giardia* infection is dependent on age (79), with some studies showing that asymptomatic *Giardia* infection is associated with growth stunting among children older than 18 months, but not infants or in children during their first 18 months (80), while others show an association between *Giardia* and growth stunting at 2 years of age (31). The effects of *Giardia* on gut microbiota may also be age

dependent. The MAL-ED study of Peruvian children found that gut microbiota associated with *Giardia* burden varied by age (35). For example, high *Giardia* burden was associated with enrichment of *Prevotella* only in fecal samples of 24-month-old children. Here, we show an association between *Giardia* infection and altered gut microbiota composition in specific age cohorts as well, raising the possibility that parasite-microbiome interactions may partially explain the age-dependent disease presentation during *Giardia* infection. Collectively, these data point to the gut microbiome, host immunity, parasite genotype, and age (81–84) as variables that may interact or operate independently to augment the balance between protection and pathogenesis during *Giardia* infection.

One major obstacle to investigating relationships between clinical variables and microbiome composition in large-scale studies like GEMS and MAL-ED is that these data are not always collected at the same time, by the same researchers, with the goal of being analyzed together. For example, although extensive clinical and epidemiological data were collected from over 22,000 participants in GEMS (30) and over 1.8 million observations in MAL-ED (34), microbiome profiling data were collected from a subset of approximately 1,000 samples from each study, and were published separately and with relatively sparse metadata (33, 38). Our study highlights that a database-driven approach that integrates microbiome data with extensive clinical and epidemiological data allows for the identification of novel associations and an opportunity to compare microbiome phenotypes across host species and across studies.

## MATERIALS AND METHODS

A dockerized environment containing code and software is available on Code Ocean (<https://codeocean.com/capsule/2815529/tree>) and fully reproduces all analyses and figures in the article.

**Canine sample collection.** Fecal samples for our Companion Animal Microbiome during Parasitism (CAMP) study were acquired from patients seen at the Ryan Hospital at the University of Pennsylvania's School of Veterinary Medicine (PennVet) as part of both sick and wellness visits, as well as from healthy dogs in animal shelters that were brought to the Ryan Hospital to be spayed or neutered. Fecal samples were examined for parasites by fecal flotation (using a zinc sulfate solution at a specific gravity of 1.18 g/ml) at the Clinical Parasitology Laboratory of PennVet. Dogs either had no observable parasites ( $n = 145$ ), one ( $n = 92$ ), or multiple ( $n = 21$ ) protozoan parasites, including *Giardia* ( $n = 32$ ) and *Cystoisospora* ( $n = 12$ ), and helminths, including hookworm (*Ancylostoma caninum*) ( $n = 19$ ), whipworm (*Trichuris vulpis*) ( $n = 12$ ), ascarid (*Toxocara canis*) ( $n = 9$ ), tapeworm (*Dipylidium caninum*) ( $n = 5$ ), and *Eucoleus boehmi* ( $n = 3$ ) (Fig. 1A). Although zinc flotation is less sensitive for detecting *Giardia* infection compared to antigen immunoassays, it has the advantage of higher specificity for detecting active *Giardia* infections rather than detecting antigen that can persist even after the infection is cleared. Samples containing yeast were excluded from the study. Age, sex, and spay and neuter status were recorded at the time of fecal sample collection for all samples. Fecal samples from 113 infected dogs and 145 dogs without detectable parasites were stored at  $-80^{\circ}\text{C}$  until DNA extraction. Clinical data from patient visits were obtained for 174 PennVet patients to determine whether gastrointestinal symptoms or antibiotic use occurred within 1 week of fecal sample collection.

**16S rRNA gene sequencing and analysis.** DNA was extracted from fecal samples using Qiagen PowerSoil DNA extraction kit. 16S rRNA gene sequencing was performed as described previously (85). Briefly, the V4 region of the 16S rRNA gene was amplified using PCR, which was performed using Accuprime Pfx supermix and custom primers for 30 cycles (85). PicoGreen quantification was used to normalize post-PCR products, and AMPureXP beads were used to clean the combined pools. Libraries were quantified and sized using a Qubit 2.0 and TapeStation 4200, respectively. Then, 250-bp paired-end sequencing was performed using an Illumina MiSeq. The QIIME2 pipeline (86) was used to process and analyze 16S sequencing data. Samples were demultiplexed using q2-demux and denoised using Dada2 (87). Sequences were aligned using mafft (88), and phylogenetic trees were reconstructed using fasttree (89). Weighted UniFrac (90) and Bray-Curtis (91) beta diversity metrics were estimated using q2-core-metrics-diversity after samples were rarefied to 4,100 reads per sample, and  $P$  values were adjusted for multiple hypothesis testing using Benjamini-Hochberg (B-H) false discovery rate (FDR) corrections (92). Taxonomy was assigned to sequences using q2-feature-classifier classify-sklearn (93) against the Greengenes 13-8 99% operational taxonomic unit (OTU) reference sequences (94). Taxa were collapsed to the genus level, when possible. OTUs with less than 1% average relative abundance across all samples were removed.

**Correlation analysis and differential feature selection.** The correlation between variables such as parasite infection and microbiota composition was determined using permutational multivariate analysis of variance (PERMANOVA) as implemented in the vegan package (95) in R (96). Differentially abundant taxa were determined using linear discriminant analysis (LDA) effect size (LEfSe) (97), and  $P$  values were adjusted for multiple hypothesis testing using B-H FDR corrections in R. Boxplots and LEfSe plots were visualized using ggplot2 (98), patchwork (99), and ggthemes (100). Point-biserial correlation coefficients were calculated to identify differentially abundant taxa between *Giardia*-infected and no parasite seen

(NPS) controls with 10,000 permutations using the *indicspecies* package in R (101), adjusting for multiple hypothesis testing using B-H FDR corrections (see Table S1 in the supplemental material).

**Integration and analysis of GEMS data.** The Global Enteric Multicenter Study (GEMS) investigated the causes, incidence, and impact of moderate to severe diarrhea in 23,567 0- to 59-month-old children in Asia and Africa (30). Clinical and epidemiological data and anthropometric measurements for each participant were downloaded from ClinEpiDB.org (36, 37). The presence of *Giardia*, *Cryptosporidium*, and rotavirus were determined using ELISA on participant fecal samples. Additionally, sequencing of the V1-V2 region of the 16S rRNA gene was performed on stool samples from 1,007 participants (33). Taxonomy was determined by classifying sequences against the Greengenes 99% OTU reference sequences. Here, clinical data from 1,004 GEMS participants was downloaded from ClinEpiDB.org, and the relative abundances of bacterial taxa for the same 1,004 participants was downloaded from MicrobiomeDB.org (102). The data sets were manually combined so that clinical and epidemiological data were matched to gut bacterial taxon abundance data.

Correlations between clinical variables (e.g., *Giardia* infection) and Bray-Curtis beta diversity were calculated using the *vegan* package in R. Patients were divided among five age groups (0 to 6, 6 to 12, 12 to 18, 18 to 24, and 24 to 59 months) to control for the effects associated with age. Here, associations with age were stratified by country, associations with country were stratified by age, and all other associations were stratified by age group and country (Fig. 5C), as done by Kotloff et al. (30). Taxonomy was collapsed to the genus level, when possible, and taxa with mean relative abundance across all samples of <1% were removed. Differentially abundant taxa between *Giardia*-positive versus *Giardia*-negative and MSD cases versus controls were determined using LEfSe, adjusting *P* values for multiple hypothesis testing using B-H FDR corrections. LEfSe plots and boxplots were visualized using *ggplot2* (98), *patchwork* (99), and *ggthemes* (100).

**Integration and analysis of MAL-ED data.** The Etiology, Risk Factors, and Interactions of Enteric Infections and Malnutrition and the Consequences for Child Health (MAL-ED) study investigated the burden of enteropathogens and malnutrition by monitoring 2,145 children across eight sites in Africa, Asia, and South America starting at 17 days of life and followed longitudinally for up to 60 months (34). Clinical and epidemiological data and anthropometric measurements for each participant were downloaded from ClinEpiDB.org (36, 37). Fecal samples were collected from participants, and the presence of *Giardia* was determined by ELISA. Additionally, sequencing of the V4 region of the 16S rRNA gene was performed on stool samples from 913 participants from the Peruvian cohort (38). Taxonomy was determined by classifying sequences against the SILVA reference database (103, 104). Here, clinical data from 913 MAL-ED observations were downloaded from ClinEpiDB.org, and the relative abundances of bacterial taxa for the same observations were downloaded from MicrobiomeDB.org (102). The data sets were manually combined so that clinical and epidemiological data were matched to gut bacterial taxa abundance data. Taxonomy was collapsed to the genus level, when possible, and taxa with mean relative abundance across all samples of <1% were removed. Differentially abundant taxa between *Giardia*-positive versus *Giardia*-negative children were determined using LEfSe, adjusting *P* values for multiple hypothesis testing using B-H FDR corrections. The effects of *Giardia* on gut microbiota composition were also stratified by stool consistency: the effects among watery or liquid stools and the effects among formed or soft stools. LEfSe plots and boxplots were visualized using *ggplot2* (98), *patchwork* (99), and *ggthemes* (100).

**Data availability.** All sequencing data analyzed here are publicly available on the Sequence Read Archive (SRA) under study accession number [PRJNA594732](https://www.ncbi.nlm.nih.gov/sra/PRJNA594732). All sequencing data used for the canine analyses is also publicly available on MicrobiomeDB.org as part of the CAMP study. All code is available and reproducible on CodeOcean (<https://codeocean.com/capsule/2815529/tree>).

## SUPPLEMENTAL MATERIAL

Supplemental material is available online only.

**FIG S1**, PDF file, 0.1 MB.

**FIG S2**, PDF file, 0.1 MB.

**FIG S3**, PDF file, 0.05 MB.

**FIG S4**, PDF file, 0.1 MB.

**FIG S5**, PDF file, 0.1 MB.

**FIG S6**, PDF file, 0.1 MB.

**TABLE S1**, PDF file, 0.04 MB.

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## REFERENCES

1. Haque R. 2007. Human intestinal parasites. *J Health Popul Nutr* 25: 387–391.
2. Menu E, Mary C, Toga I, Raoult D, Ranque S, Bittar F. 2019. A hospital qPCR-based survey of 10 gastrointestinal parasites in routine diagnostic screening, Marseille, France. *Epidemiol Infect* 147:e100. <https://doi.org/10.1017/S0950268819000165>.
3. Fletcher SM, Stark D, Harkness J, Ellis J. 2012. Enteric protozoa in the developed world: a public health perspective. *Clin Microbiol Rev* 25: 420–449. <https://doi.org/10.1128/CMR.05038-11>.
4. Levine MM, Kotloff KL, Nataro JP, Muhsen K. 2012. The Global Enteric Multicenter Study (GEMS): impetus, rationale, and genesis. *Clin Infect Dis* 55(Suppl 4):S215–S224. <https://doi.org/10.1093/cid/cis761>.
5. Moosavi A, Haghighi A, Mojarad EN, Zayeri F, Alebouyeh M, Khazan H, Kazemi B, Zali MR. 2012. Genetic variability of *Blastocystis* sp. isolated from symptomatic and asymptomatic individuals in Iran. *Parasitol Res* 111:2311–2315. <https://doi.org/10.1007/s00436-012-3085-5>.
6. Holtman GA, Kranenberg JJ, Blanker MH, Ott A, Lisman-van Leeuwen Y, Berger MY. 2017. *Dientamoeba fragilis* colonization is not associated with gastrointestinal symptoms in children at primary care level. *Fam Pract* 34:25–29. <https://doi.org/10.1093/fampra/cmw111>.
7. Jokelainen P, Hebbelstrup Jensen B, Andreassen BU, Petersen AM, Røser D, Krogfelt KA, Nielsen HV, Stensvold CR. 2017. *Dientamoeba fragilis*, a commensal in children in Danish day care centers. *J Clin Microbiol* 55:1707–1713. <https://doi.org/10.1128/JCM.00037-17>.
8. Hellard ME, Sinclair MI, Hogg GG, Fairley CK. 2000. Prevalence of enteric pathogens among community based asymptomatic individuals. *J Gastroenterol Hepatol* 15:290–293. <https://doi.org/10.1046/j.1440-1746.2000.02089.x>.
9. Reh L, Muadica AS, Köster PC, Balasegaram S, Verlander NQ, Chércoles ER, Carmena D. 2019. Substantial prevalence of enteroparasites *Cryptosporidium* spp., *Giardia duodenalis* and *Blastocystis* sp. in asymptomatic schoolchildren in Madrid, Spain, November 2017 to June 2018. *Euro Surveill* 24(43):1900241. <https://doi.org/10.2807/1560-7917.ES.2019.24.43.1900241>.
10. Wang S, El-Fahmawi A, Christian DA, Fang Q, Radaelli E, Chen L, Sullivan MC, Misis AM, Ellringer JA, Zhu X-Q, Winter SE, Hunter CA, Beiting DP. 2019. Infection-induced intestinal dysbiosis is mediated by macrophage activation and nitrate production. *mBio* 10:e00935-19. [CrossRef] <https://doi.org/10.1128/mBio.00935-19>.
11. Gimblet C, Meisel JS, Loesche MA, Cole SD, Horwinski J, Novais FO, Misis AM, Bradley CW, Beiting DP, Rankin SC, Carvalho LP, Carvalho EM, Scott P, Grice EA. 2017. Cutaneous Leishmaniasis induces a transmissible dysbiotic skin microbiota that promotes skin inflammation. *Cell Host Microbe* 22:13–24.e4. <https://doi.org/10.1016/j.chom.2017.06.006>.
12. Barash NR, Maloney JG, Singer SM, Dawson SC. 2017. *Giardia* alters commensal microbial diversity throughout the murine gut. *Infect Immun* 85:e00948-16. [CrossRef] <https://doi.org/10.1128/IAI.00948-16>.
13. Gerbaba TK, Gupta P, Rioux K, Hansen D, Buret AG. 2015. *Giardia duodenalis*-induced alterations of commensal bacteria kill *Caenorhabditis elegans*: a new model to study microbial-microbial interactions in the gut. *Am J Physiol Gastrointest Liver Physiol* 308:G550–G561. <https://doi.org/10.1152/ajpgi.00335.2014>.
14. Gerbaba TK, Green-Harrison L, Buret AG. 2017. Modeling host-microbiome interactions in *Caenorhabditis elegans*. *J Nematol* 49: 348–356.
15. Bartelt LA, Bolick DT, Mayneris-Pexachs J, Kolling GL, Medlock GL, Zaenker EI, Donowitz J, Thomas-Beckett RV, Rogala A, Carroll IM, Singer SM, Papin J, Swann JR, Guerrant RL. 2017. Cross-modulation of pathogen-specific pathways enhances malnutrition during enteric co-infection with *Giardia lamblia* and enteroaggregative *Escherichia coli*. *PLoS Pathog* 13:e1006471. <https://doi.org/10.1371/journal.ppat.1006471>.
16. Freeman CD, Klutman NE, Lamp KC. 1997. Metronidazole. A therapeutic review and update. *Drugs* 54:679–708. <https://doi.org/10.2165/00003495-199754050-00003>.
17. Jenkins TP, Peachey LE, Ajami NJ, MacDonald AS, Hsieh MH, Brindley PJ, Cantacessi C, Rinaldi G. 2018. *Schistosoma mansoni* infection is associated with quantitative and qualitative modifications of the mammalian intestinal microbiota. *Sci Rep* 8:12072. <https://doi.org/10.1038/s41598-018-30412-x>.
18. Rosa BA, Supali T, Gankpala L, Djuardi Y, Sartono E, Zhou Y, Fischer K, Martin J, Tyagi R, Bolay FK, Fischer PU, Yazdanbakhsh M, Mitreva M. 2018. Differential human gut microbiome assemblages during soil-transmitted helminth infections in Indonesia and Liberia. *Microbiome* 6:33. <https://doi.org/10.1186/s40168-018-0416-5>.
19. Renelies-Hamilton J, Noguera-Julian M, Parera M, Paredes R, Pacheco L, Dacal E, Saugar JM, Rubio JM, Poulsen M, Köster PC, Carmena D. 2019. Exploring interactions between *Blastocystis* sp., *Strongyloides* spp. and the gut microbiomes of wild chimpanzees in Senegal. *Infect Genet Evol* 74:104010. <https://doi.org/10.1016/j.meegid.2019.104010>.
20. Gates MC, Nolan TJ. 2009. Endoparasite prevalence and recurrence across different age groups of dogs and cats. *Vet Parasitol* 166:153–158. <https://doi.org/10.1016/j.vetpar.2009.07.041>.
21. Little SE, Johnson EM, Lewis D, Jaklitsch RP, Payton ME, Blagburn BL, Bowman DD, Moroff S, Tams T, Rich L, Aucoin D. 2009. Prevalence of intestinal parasites in pet dogs in the United States. *Vet Parasitol* 166:144–152. <https://doi.org/10.1016/j.vetpar.2009.07.044>.
22. Wang S, Martins R, Sullivan MC, Friedman ES, Misis AM, El-Fahmawi A, De Martinis ECP, O'Brien K, Chen Y, Bradley C, Zhang G, Berry ASF, Hunter CA, Baldassano RN, Rondeau MP, Beiting DP. 2019. Diet-induced remission in chronic enteropathy is associated with altered microbial community structure and synthesis of secondary bile acids. *Microbiome* 7:126. <https://doi.org/10.1186/s40168-019-0740-4>.
23. Cerquetella M, Spaterna A, Laus F, Tesi B, Rossi G, Antonelli E, Villanacci V, Bassotti G. 2010. Inflammatory bowel disease in the dog: differences and similarities with humans. *World J Gastroenterol* 16: 1050–1056. <https://doi.org/10.3748/wjg.v16.i9.1050>.
24. Jergens AE, Simpson KW. 2012. Inflammatory bowel disease in veterinary medicine. *Front Biosci (Elite Ed)* 4:1404–1419. <https://doi.org/10.2741/470>.
25. Peiravan A, Bertolini F, Rothschild MF, Simpson KW, Jergens AE, Allenspach K, Werling D. 2018. Genome-wide association studies of inflammatory bowel disease in German shepherd dogs. *PLoS One* 13: e0200685. <https://doi.org/10.1371/journal.pone.0200685>.
26. Vázquez-Baeza Y, Hyde ER, Suchodolski JS, Knight R. 2016. Dog and human inflammatory bowel disease rely on overlapping yet distinct dysbiosis networks. *Nat Microbiol* 1:16177. <https://doi.org/10.1038/nmicrobiol.2016.177>.
27. Suchodolski JS, Markel ME, Garcia-Mazcorro JF, Unterer S, Heilmann RM, Dowd SE, Kachroo P, Ivanov I, Minamoto Y, Dillman EM, Steiner JM, Cook AK, Toresson L. 2012. The fecal microbiome in dogs with acute diarrhea and idiopathic inflammatory bowel disease. *PLoS One* 7:e51907. <https://doi.org/10.1371/journal.pone.0051907>.
28. Simpson KW, Dogan B, Rishniw M, Goldstein RE, Klaessig S, McDonough PL, German AJ, Yates RM, Russell DG, Johnson SE, Berg DE, Harel J, Bruant G, McDonough SP, Schukken YH. 2006. Adherent and invasive *Escherichia coli* is associated with granulomatous colitis in boxer dogs. *Infect Immun* 74:4778–4792. <https://doi.org/10.1128/IAI.00067-06>.
29. Coelho LP, Kultima JR, Costea PI, Fournier C, Pan Y, Czarnecki-Maulden G, Hayward MR, Forslund SK, Schmidt TSB, Descombes P, Jackson JR, Li Q, Bork P. 2018. Similarity of the dog and human gut microbiomes in gene content and response to diet. *Microbiome* 6:72. <https://doi.org/10.1186/s40168-018-0450-3>.
30. Kotloff KL, Nataro JP, Blackwelder WC, Nasrin D, Farag TH, Panchalingam S, Wu Y, Sow SO, Sur D, Breiman RF, Faruque AS, Zaidi AK, Saha D, Alonso PL, Tamboura B, Sanogo D, Onwuchekwa U, Manna B, Ramamurthy T, Kanungo S, Ochieng JB, Omore R, Oundo JO, Hossain A, Das SK, Ahmed S, Qureshi S, Quadri F, Adegbola RA, Antonio M, Hossain MJ, Akinsola A, Mandomando I, Nhampossa T, Acácio S, Biswas K, O'Reilly CE, Mintz ED, Berkeley LY, Muhsen K, Sommerfelt H, Robins-Browne RM, Levine MM. 2013. Burden and aetiology of diarrhoeal disease in infants and young children in developing countries (the Global Enteric Multicenter Study, GEMS): a prospective, case-control study. *Lancet* 382:209–222. [https://doi.org/10.1016/S0140-6736\(13\)60844-2](https://doi.org/10.1016/S0140-6736(13)60844-2).
31. Rogawski ET, Liu J, Platts-Mills JA, Kabir F, Lertsethtakarn P, Sigauz M, Khan SS, Praharaj I, Murei A, Nshama R, Mujaga B, Havt A, Maciel IA, Operario DJ, Taniuchi M, Gratz J, Stroup SE, Roberts JH, Kalam A, Aziz F, Qureshi S, Islam MO, Sakpaial P, Silapong S, Yori PP, Rajendiran R, Benny B, McGrath M, Seidman JC, Lang D, Gottlieb M, Guerrant RL, Lima AAM, Leite JP, Samie A, Bessong PO, Page N, Bodhidatta L, Mason C, Shrestha S, Kiwelu I, Mduma ER, Iqbal NT, Bhutta ZA, Ahmed T, Haque



- R, Kang G, Kosek MN, Houpt ER, MAL-ED Network Investigators. 2018. Use of quantitative molecular diagnostic methods to investigate the effect of enteropathogen infections on linear growth in children in low-resource settings: longitudinal analysis of results from the MAL-ED cohort study. *Lancet Glob Health* 6:e1319–e1328. [https://doi.org/10.1016/S2214-109X\(18\)30351-6](https://doi.org/10.1016/S2214-109X(18)30351-6).
32. Liteskare S, Rortveit G, Eide GE, Emberland KE, Hanevik K, Langeland N, Wensaas K-A. 2019. Quality of life and its association with irritable bowel syndrome and fatigue ten years after giardiasis. *Neurogastroenterol Motil* 31:e13559. <https://doi.org/10.1111/nmo.13559>.
33. Pop M, Walker AW, Paulson J, Lindsay B, Antonio M, Hossain MA, Oundo J, Tamboura B, Mai V, Astrovskaya I, Corrada Bravo H, Rance R, Stares M, Levine MM, Panchalingam S, Kotloff K, Ikumapayi UN, Ebruke C, Adeyemi M, Ahmed D, Ahmed F, Alam MT, Amin R, Siddiqui S, Ochieng JB, Ouma E, Juma J, Mailu E, Omoro R, Morris JG, Breiman RF, Saha D, Parkhill J, Nataro JP, Stine OC. 2014. Diarrhea in young children from low-income countries leads to large-scale alterations in intestinal microbiota composition. *Genome Biol* 15:R76. <https://doi.org/10.1186/gb-2014-15-6-r76>.
34. MAL-ED Network Investigators. 2014. The MAL-ED study: a multinational and multidisciplinary approach to understand the relationship between enteric pathogens, malnutrition, gut physiology, physical growth, cognitive development, and immune responses in infants and children up to 2 years of age in resource-poor environments. *Clin Infect Dis* 59(Suppl 4):S193–S206. <https://doi.org/10.1093/cid/ciu653>.
35. Rouhani S, Griffin NW, Yori PP, Olortegui MP, Sigalas Salas M, Rengifo Trigos D, Moulton LH, Houpt ER, Barratt MJ, Kosek MN, Gordon JL. 2019. Gut microbiota features associated with campylobacter burden and postnatal linear growth deficits in a Peruvian birth cohort. *Clin Infect Dis* <https://doi.org/10.1093/cid/ciz906>.
36. Aurrecochea C, Barreto A, Basenko EY, Brestelli J, Brunk BP, Cade S, Crouch K, Doherty R, Falke D, Fischer S, Gajria B, Harb OS, Heiges M, Hertz-Fowler C, Hu S, Iodice J, Kissinger JC, Lawrence C, Li W, Pinney DF, Pulman JA, Roos DS, Shanmugasundaram A, Silva-Franco F, Steinbiss S, Stoeckert CJ, Spruill D, Wang H, Warrenfeltz S, Zheng J. 2017. EuPathDB: the eukaryotic pathogen genomics database resource. *Nucleic Acids Res* 45:D581–D591. <https://doi.org/10.1093/nar/gkw1105>.
37. Ruhamyankaka E, Brunk BP, Dorsey G, Harb OS, Helb DA, Judkins J, Kissinger JC, Lindsay BR, Roos DS, Stoeckert CJ, Zheng J, Shah Tomko S. 2019. ClinEpiDB: an open-access clinical epidemiology database resource encouraging online exploration of complex studies. *Zenodo* <https://doi.org/10.5281/zenodo.3522209>.
38. Rouhani S, Griffin NW, Yori PP, Gehrig JL, Olortegui MP, Salas MS, Trigos DR, Moulton LH, Houpt ER, Barratt MJ, Kosek MN, Gordon JL. 2019. Diarrhea as a potential cause and consequence of reduced gut microbial diversity among undernourished children in Peru. *Clin Infect Dis* <https://doi.org/10.1093/cid/ciz905>.
39. Kostic AD, Xavier RJ, Gevers D. 2014. The microbiome in inflammatory bowel disease: current status and the future ahead. *Gastroenterology* 146:1489–1499. <https://doi.org/10.1053/j.gastro.2014.02.009>.
40. Torres J, Hu J, Seki A, Eisele C, Nair N, Huang R, Tarassishin L, Jharap B, Cote-Daigneault J, Mao Q, Mogno I, Britton G, Uzzan M, Chen C-L, Kornbluth A, George J, Legnani P, Maser E, Loudon H, Stone J, Dubinsky M, Faith JJ, Clemente JC, Mehandru S, Colombel J-F, Peter I. 2020. Infants born to mothers with IBD present with altered gut microbiome that transfers abnormalities of the adaptive immune system to germ-free mice. *Gut* 69:42–51. <https://doi.org/10.1136/gutjnl-2018-317855>.
41. Heidarian F, Alebouyeh M, Shahrokh S, Balaii H, Zali MR. 2019. Altered fecal bacterial composition correlates with disease activity in inflammatory bowel disease and the extent of IL8 induction. *Curr Res Transl Med* 67:41–50. <https://doi.org/10.1016/j.retram.2019.01.002>.
42. Holm JB, Sorobetea D, Kiilerich P, Ramayo-Caldas Y, Estellé J, Ma T, Madsen L, Kristiansen K, Svensson-Frej M. 2015. Chronic *Trichuris muris* infection decreases diversity of the intestinal microbiota and concomitantly increases the abundance of *Lactobacilli*. *PLoS One* 10:e0125495. <https://doi.org/10.1371/journal.pone.0125495>.
43. Houlden A, Hayes KS, Bancroft AJ, Worthington JJ, Wang P, Grencis RK, Roberts IS. 2015. Chronic *Trichuris muris* infection in C57BL/6 mice causes significant changes in host microbiota and metabolome: effects reversed by pathogen clearance. *PLoS One* 10:e0125945. <https://doi.org/10.1371/journal.pone.0125945>.
44. Cattadori IM, Sebastian A, Hao H, Katani R, Albert I, Eilertson KE, Kapur V, Pathak A, Mitchell S. 2016. Impact of helminth infections and nutritional constraints on the small intestine microbiota. *PLoS One* 11:e0159770. <https://doi.org/10.1371/journal.pone.0159770>.
45. Lee SC, Tang MS, Easton AV, Devlin JC, Chua LL, Cho I, Moy FM, Khang TF, Lim YAL, Loke P. 2019. Linking the effects of helminth infection, diet and the gut microbiota with human whole-blood signatures. *PLoS Pathog* 15:e1008066. <https://doi.org/10.1371/journal.ppat.1008066>.
46. Toro-Londono MA, Bedoya-Urrego K, Garcia-Montoya GM, Galvan-Diaz AL, Alzate JF. 2019. Intestinal parasitic infection alters bacterial gut microbiota in children. *PeerJ* 7:e6200. <https://doi.org/10.7717/peerj.6200>.
47. Cooper P, Walker AW, Reyes J, Chico M, Salter SJ, Vaca M, Parkhill J. 2013. Patent human infections with the whipworm, *Trichuris trichiura*, are not associated with alterations in the faecal microbiota. *PLoS One* 8:e76573. <https://doi.org/10.1371/journal.pone.0076573>.
48. Schneeberger PHH, Coulbaly JT, Panic G, Daubenberger C, Gueuning M, Frey JE, Keiser J. 2018. Investigations on the interplays between *Schistosoma mansoni*, praziquantel and the gut microbiome. *Parasit Vectors* 11:168. <https://doi.org/10.1186/s13071-018-2739-2>.
49. Lee NN, Bidot WA, Ericsson AC, Franklin CL. 2020. Effects of *Giardia lamblia* colonization and fenbendazole treatment on canine fecal microbiota. *J Am Assoc Lab Anim Sci* 59:423–429. <https://doi.org/10.30802/AALAS-JAALAS-19-000113>.
50. Fujishiro MA, Lidbury JA, Pilla R, Steiner JM, Lappin MR, Suchodolski JS. 2020. Evaluation of the effects of anthelmintic administration on the fecal microbiome of healthy dogs with and without subclinical *Giardia* spp. and *Cryptosporidium canis* infections. *PLoS One* 15:e0228145. <https://doi.org/10.1371/journal.pone.0228145>.
51. Muhsen K, Levine MM. 2012. A systematic review and meta-analysis of the association between *Giardia lamblia* and endemic pediatric diarrhea in developing countries. *Clin Infect Dis* 55(Suppl 4):S271–S293. <https://doi.org/10.1093/cid/cis762>.
52. Robertson ID, Thompson RC. 2002. Enteric parasitic zoonoses of domesticated dogs and cats. *Microbes Infect* 4:867–873. [https://doi.org/10.1016/S1286-4579\(02\)01607-6](https://doi.org/10.1016/S1286-4579(02)01607-6).
53. Liu J, Kabir F, Manneh J, Lertsethtakarn P, Begum S, Gratz J, Becker SM, Operario DJ, Taniuchi M, Janaki L, Platts-Mills JA, Haverstick DM, Kabir M, Sobuz SU, Nakjarung K, Sakpaisal P, Silapong S, Bodhidatta L, Qureshi S, Kalam A, Saidi Q, Swai N, Mujaga B, Maro A, Kwambana B, Dione M, Antonio M, Kibiki G, Mason CJ, Haque R, Iqbal N, Zaidi AKM, Houpt ER. 2014. Development and assessment of molecular diagnostic tests for 15 enteropathogens causing childhood diarrhoea: a multicentre study. *Lancet Infect Dis* 14:716–724. [https://doi.org/10.1016/S1473-3099\(14\)70808-4](https://doi.org/10.1016/S1473-3099(14)70808-4).
54. Breurec S, Vanel N, Bata P, Chartier L, Farra A, Favennec L, Franck T, Giles-Vernick T, Gody J-C, Luong Nguyen LB, Onambélé M, Rafai C, Razakandrainibe R, Tondeur L, Tricou V, Sansonetti P, Vray M. 2016. Etiology and epidemiology of diarrhea in hospitalized children from low income country: a matched case-control study in Central African Republic. *PLoS Negl Trop Dis* 10:e0004283. <https://doi.org/10.1371/journal.pntd.0004283>.
55. Platts-Mills JA, Babji S, Bodhidatta L, Gratz J, Haque R, Havt A, McCormick BJ, McGrath M, Olortegui MP, Samie A, Shakoor S, Mondal D, Lima IF, Hariraju D, Rayamajhi BB, Qureshi S, Kabir F, Yori PP, Mufamadi B, Amour C, Carreon JD, Richard SA, Lang D, Bessong P, Mduma E, Ahmed T, Lima AA, Mason CJ, Zaidi AK, Bhutta ZA, Kosek M, Guerrant RL, Gottlieb M, Miller M, Kang G, Houpt ER, MAL-ED Network Investigators. 2015. Pathogen-specific burdens of community diarrhoea in developing countries: a multisite birth cohort study (MAL-ED). *Lancet Glob Health* 3:e564–e575. [https://doi.org/10.1016/S2214-109X\(15\)00151-5](https://doi.org/10.1016/S2214-109X(15)00151-5).
56. Bartelt LA, Platts-Mills JA. 2016. *Giardia*: a pathogen or commensal for children in high-prevalence settings? *Curr Opin Infect Dis* 29:502–507. <https://doi.org/10.1097/QCO.0000000000000293>.
57. Tysnes KR, Skancke E, Robertson LJ. 2014. Subclinical *Giardia* in dogs: a veterinary conundrum relevant to human infection. *Trends Parasitol* 30:520–527. <https://doi.org/10.1016/j.pt.2014.08.007>.
58. Covacin C, Aucoin DP, Elliot A, Thompson RCA. 2011. Genotypic characterisation of *Giardia* from domestic dogs in the USA. *Vet Parasitol* 177:28–32. <https://doi.org/10.1016/j.vetpar.2010.11.029>.
59. Muhsen K, Cohen D, Levine MM. 2014. Can *Giardia lamblia* infection lower the risk of acute diarrhea among preschool children? *J Trop Pediatr* 60:99–103. <https://doi.org/10.1093/tropej/fmt085>.
60. Veenemans J, Mank T, Ottenhof M, Baidjoe A, Mbugi EV, Demir AY, Wielders JPM, Savelkoul HFJ, Verhoef H. 2011. Protection against diarrhea associated with *Giardia intestinalis* is lost with multi-nutrient

- supplementation: a study in Tanzanian children. *PLoS Negl Trop Dis* 5:e1158. <https://doi.org/10.1371/journal.pntd.0001158>.
61. Roxström-Lindquist K, Palm D, Reiner D, Ringqvist E, Svård SG. 2006. Giardia immunity—an update. *Trends Parasitol* 22:26–31. <https://doi.org/10.1016/j.pt.2005.11.005>.
  62. Manko-Prykhoda A, Allain T, Motta J-P, Cotton JA, Feener T, Oyeyemi A, Bindra S, Vallance BA, Wallace JL, Beck P, Buret AG. 2020. Giardia spp. promote the production of antimicrobial peptides and attenuate disease severity induced by attaching and effacing enteropathogens via the induction of the NLRP3 inflammasome. *Int J Parasitol* 50:263–275. <https://doi.org/10.1016/j.ijpara.2019.12.011>.
  63. Mejia R, Damania A, Jeun R, Bryan PE, Vargas P, Juarez M, Cajal PS, Nasser J, Krolewiecki A, Lefoulon E, Long C, Drake E, Cimino RO, Slatko B. 2020. Impact of intestinal parasites on microbiota and cobalamin gene sequences: a pilot study. *Parasit Vectors* 13:200. <https://doi.org/10.1186/s13071-020-04073-7>.
  64. Nieves-Ramírez ME, Partida-Rodríguez O, Laforest-Lapointe I, Reynolds LA, Brown EM, Valdez-Salazar A, Morán-Silva P, Rojas-Velázquez L, Morien E, Parfrey LW, Jin M, Walter J, Torres J, Arrieta MC, Ximénez-García C, Finlay BB. 2018. Asymptomatic intestinal colonization with protist blastocystis is strongly associated with distinct microbiome ecological patterns. *mSystems* 3:e00007-18. [CrossRef] <https://doi.org/10.1128/mSystems.00007-18>.
  65. Shaulov Y, Shimokawa C, Trebic-Geffen M, Nagaraja S, Methling K, Lalk M, Weiss-Cerem L, Lamm AT, Hiseada H, Ankri S. 2018. Escherichia coli mediated resistance of Entamoeba histolytica to oxidative stress is triggered by oxaloacetate. *PLoS Pathog* 14:e1007295. <https://doi.org/10.1371/journal.ppat.1007295>.
  66. Hayes KS, Bancroft AJ, Goldrick M, Portsmouth C, Roberts IS, Grencis RK. 2010. Exploitation of the intestinal microflora by the parasitic nematode Trichuris muris. *Science* 328:1391–1394. <https://doi.org/10.1126/science.1187703>.
  67. Singer SM, Nash TE. 2000. The role of normal flora in Giardia lamblia infections in mice. *J Infect Dis* 181:1510–1512. <https://doi.org/10.1086/315409>.
  68. Farthing MJG. 1993. Pathogenesis of giardiasis. *Trans R Soc Trop Med Hyg* 87:17–21. [https://doi.org/10.1016/0035-9203\(93\)90531-T](https://doi.org/10.1016/0035-9203(93)90531-T).
  69. Hartong WA, Gourley WK, Arvanitakis C. 1979. Giardiasis: clinical spectrum and functional-structural abnormalities of the small intestinal mucosa. *Gastroenterology* 77:61–69. [https://doi.org/10.1016/S0016-5085\(79\)80011-6](https://doi.org/10.1016/S0016-5085(79)80011-6).
  70. Khanna R, Vinayak VK, Mehta S, Kumkum, Nain CK. 1988. Giardia lamblia infection in immunosuppressed animals causes severe alterations to brush border membrane enzymes. *Dig Dis Sci* 33:1147–1152. <https://doi.org/10.1007/BF01535792>.
  71. Beatty JK, Akierman SV, Motta J-P, Muise S, Workentine ML, Harrison JJ, Bhargava A, Beck PL, Rioux KP, McKnight GW, Wallace JL, Buret AG. 2017. Giardia duodenalis induces pathogenic dysbiosis of human intestinal microbiota biofilms. *Int J Parasitol* 47:311–326. <https://doi.org/10.1016/j.ijpara.2016.11.010>.
  72. Buret AG, Motta J-P, Allain T, Ferraz J, Wallace JL. 2019. Pathobiont release from dysbiotic gut microbiota biofilms in intestinal inflammatory diseases: a role for iron? *J Biomed Sci* 26:1. <https://doi.org/10.1186/s12929-018-0495-4>.
  73. Soriano-Arandes A, García-Carrasco E, Serre-Delcor N, Treviño-Maruri B, Sulleiro E, Ruiz-Giardin JM, Sanmartín JV, Torrús D, Rojo-Marcos G, Cuadros J, Martín-Echevarría E, López-Vélez R, Molina I, Pérez-Molina JA, Redivi Study Group. 2016. Travelers' diarrhea in children at risk: an observational study from a Spanish database. *Pediatr Infect Dis J* 35:392–395. <https://doi.org/10.1097/INF.0000000000001049>.
  74. Cacciò SM, Ryan U. 2008. Molecular epidemiology of giardiasis. *Mol Biochem Parasitol* 160:75–80. <https://doi.org/10.1016/j.molbiopara.2008.04.006>.
  75. Haque R, Roy S, Kabir M, Stroup SE, Mondal D, Houpt ER. 2005. Giardia assemblage A infection and diarrhea in Bangladesh. *J Infect Dis* 192: 2171–2173. <https://doi.org/10.1086/498169>.
  76. Eckmann L. 2003. Mucosal defences against Giardia. *Parasite Immunol* 25:259–270. <https://doi.org/10.1046/j.1365-3024.2003.00634.x>.
  77. Homan WL, Mank TG. 2001. Human giardiasis: genotype linked differences in clinical symptomatology. *Int J Parasitol* 31:822–826. [https://doi.org/10.1016/S0020-7519\(01\)00183-7](https://doi.org/10.1016/S0020-7519(01)00183-7).
  78. Andersson T, Forssell J, Sterner G. 1972. Outbreak of giardiasis: effect of a new antiflagellate drug, tinidazole. *Br Med J* 2:449–451. <https://doi.org/10.1136/bmj.2.5811.449>.
  79. Donowitz JR, Alam M, Kabir M, Ma JZ, Nazib F, Platts-Mills JA, Bartelt LA, Haque R, Petri WA. 2016. A prospective longitudinal cohort to investigate the effects of early life giardiasis on growth and all cause diarrhea. *Clin Infect Dis* 63:792–797. <https://doi.org/10.1093/cid/ciw391>.
  80. Lehto K-M, Fan Y-M, Oikarinen S, Nurminen N, Hallamaa L, Juuti R, Mangani C, Maleta K, Hyöty H, Ashorn P. 2019. Presence of Giardia lamblia in stools of six- to 18-month old asymptomatic Malawians is associated with children's growth failure. *Acta Paediatr* 108:1833–1840. <https://doi.org/10.1111/apa.14832>.
  81. Biagi E, Nylund L, Candela M, Ostan R, Bucci L, Pini E, Nikkila J, Monti D, Satokari R, Franceschi C, Brigidi P, De Vos W. 2010. Through ageing, and beyond: gut microbiota and inflammatory status in seniors and centenarians. *PLoS One* 5:e10667. <https://doi.org/10.1371/journal.pone.0010667>.
  82. Shulzhenko N, Morgun A, Hsiao W, Battle M, Yao M, Gavrilova O, Orandle M, Mayer L, Macpherson AJ, McCoy KD, Fraser-Liggett C, Matzinger P. 2011. Crosstalk between B lymphocytes, microbiota and the intestinal epithelium governs immunity versus metabolism in the gut. *Nat Med* 17:1585–1593. <https://doi.org/10.1038/nm.2505>.
  83. Kolling G, Wu M, Guerrant RL. 2012. Enteric pathogens through life stages. *Front Cell Infect Microbiol* 2:114. <https://doi.org/10.3389/fcimb.2012.00114>.
  84. Sahagún J, Clavel A, Goñi P, Seral C, Llorente MT, Castillo FJ, Capilla S, Arias A, Gómez-Lus R. 2008. Correlation between the presence of symptoms and the Giardia duodenalis genotype. *Eur J Clin Microbiol Infect Dis* 27:81–83. <https://doi.org/10.1007/s10096-007-0404-3>.
  85. Kozich JJ, Westcott SL, Baxter NT, Highlander SK, Schloss PD. 2013. Development of a dual-index sequencing strategy and curation pipeline for analyzing amplicon sequence data on the MiSeq Illumina sequencing platform. *Appl Environ Microbiol* 79:5112–5120. <https://doi.org/10.1128/AEM.01043-13>.
  86. Bolyen E, Rideout JR, Dillon MR, Bokulich NA, Abnet CC, Al-Ghalith GA, Alexander H, Alm EJ, Arumugam M, Asnicar F, Bai Y, Bisanz JE, Bittinger K, Brejnrod A, Brislawn CJ, Brown CT, Callahan BJ, Caraballo-Rodríguez AM, Chase J, Cope EK, Da Silva R, Diener C, Dorrestein PC, Douglas GM, Durall DM, Duvallet C, Edwardson CF, Ernst M, Estaki M, Fouquier J, Gauglitz JM, Gibbons SM, Gibson DL, Gonzalez A, Gorlick K, Guo J, Hillmann B, Holmes S, Holste H, Huttenhower C, Huttley GA, Janssen S, Jarmusch AK, Jiang L, Kaehler BD, Kang KB, Keefe CR, Keim P, Kelley ST, Knights D, Koester I, et al. 2019. Reproducible, interactive, scalable and extensible microbiome data science using QIIME 2. *Nat Biotechnol* 37:852–857. <https://doi.org/10.1038/s41587-019-0209-9>.
  87. Callahan BJ, McMurdie PJ, Rosen MJ, Han AW, Johnson AJA, Holmes SP. 2016. DADA2: high-resolution sample inference from Illumina amplicon data. *Nat Methods* 13:581–583. <https://doi.org/10.1038/nmeth.3869>.
  88. Katoh K, Misawa K, Kuma K, Miyata T. 2002. MAFFT: a novel method for rapid multiple sequence alignment based on fast Fourier transform. *Nucleic Acids Res* 30:3059–3066. <https://doi.org/10.1093/nar/gkf436>.
  89. Price MN, Dehal PS, Arkin AP. 2010. FastTree 2 — approximately maximum-likelihood trees for large alignments. *PLoS One* 5:e9490. <https://doi.org/10.1371/journal.pone.0009490>.
  90. Lozupone CA, Hamady M, Kelley ST, Knight R. 2007. Quantitative and qualitative beta diversity measures lead to different insights into factors that structure microbial communities. *Appl Environ Microbiol* 73: 1576–1585. <https://doi.org/10.1128/AEM.01996-06>.
  91. Sorensen T. 1948. A method of establishing groups of equal amplitude in plant sociology based on similarity of species content and its application to analyses of vegetation on Danish Commons. *Kongelige Danske Videnskabsbernes Selskab, Biologiske Skrifter Bind 5, Nr. 4*.
  92. Benjamini Y, Hochberg Y. 1995. Controlling the false discovery rate: a practical and powerful approach to multiple testing. *J R Stat Soc Series B (Methodological)* 57:289–300. <https://doi.org/10.1111/j.2517-6161.1995.tb02031.x>.
  93. Bokulich NA, Kaehler BD, Rideout JR, Dillon M, Bolyen E, Knight R, Huttley GA, Caporaso JG. 2018. Optimizing taxonomic classification of marker-gene amplicon sequences with QIIME 2's q2-feature-classifier plugin. *Microbiome* 6:90. <https://doi.org/10.1186/s40168-018-0470-z>.
  94. McDonald D, Price MN, Goodrich J, Nawrocki EP, DeSantis TZ, Probst A, Andersen GL, Knight R, Hugenholtz P. 2012. An improved Greengenes taxonomy with explicit ranks for ecological and evolutionary analyses of bacteria and archaea. *ISME J* 6:610–618. <https://doi.org/10.1038/ismej.2011.139>.
  95. Oksanen J, Blanchet FG, Friendly M, Kindt R, Legendre P, McGlinn D, Minchin PR, O'Hara RB, Simpson GL, Solymos P, Stevens MHH, Szöcs E,

- Wagner H. 2019. vegan: community ecology package. Computer software, R.
96. R Core Team. 2018. R: a language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria.
  97. Segata N, Izard J, Waldron L, Gevers D, Miropolsky L, Garrett WS, Huttenhower C. 2011. Metagenomic biomarker discovery and explanation. *Genome Biol* 12:R60. <https://doi.org/10.1186/gb-2011-12-6-r60>.
  98. Wickham H. 2016. ggplot2 — elegant graphics for data analysis. Springer-Verlag, New York, NY.
  99. Pedersen TL. 2017. patchwork: the composer of ggplots. Computer software, R.
  100. Arnold JB. 2019. ggthemes: extra themes, scales and geoms for “ggplot2.” Computer software, R.
  101. De Cáceres M, Legendre P. 2009. Associations between species and groups of sites: indices and statistical inference. *Ecology* 90:3566–3574. <https://doi.org/10.1890/08-1823.1>.
  102. Oliveira FS, Brestelli J, Cade S, Zheng J, Iodice J, Fischer S, Aurrecochea C, Kissinger JC, Brunk BP, Stoeckert CJ, Fernandes GR, Roos DS, Beiting DP. 2018. MicrobiomeDB: a systems biology platform for integrating, mining and analyzing microbiome experiments. *Nucleic Acids Res* 46:D684–D691. <https://doi.org/10.1093/nar/gkx1027>.
  103. Quast C, Pruesse E, Yilmaz P, Gerken J, Schweer T, Yarza P, Peplies J, Glöckner FO. 2013. The SILVA ribosomal RNA gene database project: improved data processing and web-based tools. *Nucleic Acids Res* 41:D590–D596. <https://doi.org/10.1093/nar/gks1219>.
  104. Yilmaz P, Parfrey LW, Yarza P, Gerken J, Pruesse E, Quast C, Schweer T, Peplies J, Ludwig W, Glöckner FO. 2014. The SILVA and “All-species Living Tree Project (LTP)” taxonomic frameworks. *Nucleic Acids Res* 42:D643–D648. <https://doi.org/10.1093/nar/gkt1209>.
  105. Hansen MEB, Rubel MA, Bailey AG, Ranciaro A, Thompson SR, Campbell MC, Beggs W, Dave JR, Mokone GG, Mpoloka SW, Nyambo T, Abnet C, Chanock SJ, Bushman FD, Tishkoff SA. 2019. Population structure of human gut bacteria in a diverse cohort from rural Tanzania and Botswana. *Genome Biol* 20:16. <https://doi.org/10.1186/s13059-018-1616-9>.