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Giardiasis and diarrhea in dogs: Does the microbiome matter?

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Abstract

Background: *Giardia duodenalis* (Gd) causes intestinal parasitosis. The involvement of the intestinal microbiome in determining the infection's clinical phenotype is unknown.

Objective: Investigate the fecal microbiome features in dogs with giardiasis.

Animals and Methods: Cross-sectional study, including fecal samples of kenneled dogs with Gd diagnosed by fecal *Giardia* antigen dot ELISA. The fecal microbial compositional characteristics and dysbiosis index (DI) were compared between diarrheic and nondiarrheic dogs.

Results: Fecal samples of 38 Gd-infected dogs (diarrheic, 21; nondiarrheic, 17) were included. No differences were found in Faith's phylogenic diversity and beta diversity (weighted UniFrac distances) and in specific taxa abundances at the phylum, genus, and species levels, as well as in alpha and beta diversities between diarrheic and nondiarrheic dogs, and also when divided by sex or age. Among diarrheic dogs, alpha diversity was higher in males than in females (pairwise Kruskal-Wallis, $q = 0.01$). Among males, fecal abundances of the genus *Clostridium* ($W = 19$) and *Clostridium spiroforme* species ($W = 33$) were higher in diarrheic compared to nondiarrheic dogs. In diarrheic dog fecal samples, *Proteobacteria* were more prevalent ($W = 1$), whereas *Verrucomicrobia* were less prevalent in dogs <1 year of age than in older dogs. The fecal sample DI of 19 diarrheic and 19 nondiarrheic dogs was similar (median, −0.2; range, −4.3 to 4.5 and median, −1.0; range, −4.3 to 5.8, respectively).

Abbreviations: ANCOM, analysis of microbiome composition; DI, dysbiosis index; Faith's PD, Faith's phylogenetic diversity; Gd, *Giardia duodenalis*.

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Conclusions: The fecal microbial composition of symptomatic and asymptomatic dogs with giardiasis is similar. Based on fecal DI, giardiasis is not characterized by prominent dysbiosis. Other host and parasite characteristics might determine the severity of giardiasis in dogs.

KEYWORDS
canine, *Clostridium*, dysbiosis index, *Giardia duodenalis*, *proteobacteria*, *Verrucomicrobia*

1 | INTRODUCTION

Giardia duodenalis (Gd) is an extracellular protozoan intestinal parasite, infecting several animal species and humans. Giardiasis is the most common acute or chronic parasitic diarrheal disease in dogs worldwide.[1,2] The clinical severity of Gd infection varies from an asymptomatic healthy carrier state to severe diarrhea with malabsorption, especially in young or immunosuppressed dogs.[3,4] With its zoonotic potential, Gd infection has public health importance,[5,6] and it is the most common intestinal parasitic disease worldwide.[7-9]

The clinical relevance of giardiasis remains controversial despite high infection rates, because most infected hosts are asymptomatic.[10,11] Nevertheless, certain hosts can develop diarrheal giardiasis, sustaining chronic infection.[1,3,12,13] Secondary postinfectious syndromes, including irritable bowel syndrome and food allergy, persisting beyond detectable parasite fecal shedding might occur in humans, for unknown reasons.[14-17] Lastly, resistance of Gd to conventional treatment (eg, fenbendazole, metronidazole) is increasingly reported, warranting investigation into its virulence and resistance mechanisms, host immune response and alternative effective treatment.[13]

The intestinal barrier, composed of the intestinal microbiome, mucus, and epithelial lining, is disrupted by Gd, thereby initiating the pathophysiology of giardiasis.[18] Inherent virulence factors contribute to the severity of giardiasis. Cysteine proteases disrupt intestinal epithelial apical junctional complexes, mediate mucin depletion, degrade host immune factors and break down microbiota biofilms, subsequently inducing pathogenic transformation of commensal organisms.[19] Several host and environmental factors also modulate the outcome of the disease, including immune factors, age, environmental stress, and concurrent infections.[3,4,20-22]

The Gd-gut microbiota crosstalk has garnered interest, both as a component of disease pathogenesis and as a potential therapeutic target.[23] Commensal microbiota control gut colonization and establishment of Gd.[23] Protective anti-*Giardia* microbiota can be effectively transferred and prevent Gd establishment in mice.[24] Additionally, dysbiosis contributes to giardiasis-associated clinical signs during acute giardiasis in mice and humans.[18,21,25] Dogs naturally infected with Gd have increased gut microbiota diversity.[22] Nevertheless, the latter was compared between 5 asymptomatic and 5 symptomatic dogs only, limiting the conclusions that can be made regarding the intestinal microbiota's role in the severity of giardiasis in dogs.[22] An additional study in dogs and humans reported that Gd infection is associated with significant gut microbiome remodeling, but detected changes often were positively associated with gut health, possibly accounting for the high prevalence of asymptomatic Gd infection.[26] Furthermore, significant beta diversity differences between Gd-infected and healthy dogs were detected, but were rather minimal, with large overlap between groups.[26] Finally, the latter findings were based on a study of a small heterogeneous cohort (13 dogs), with differences in habitats, ages and historical antibiotic treatments, limiting general conclusions regarding microbiome-mediated effects on host health and the clinical phenotype of giardiasis.[26]

Our aim was to investigate potential associations between fecal microbiome characteristics in a relatively large and homogeneous (in terms of age and habitat) cohort of dogs naturally infected with Gd, with and without diarrhea, and evaluate the associations between fecal microbiome features and gastrointestinal clinical signs in giardiasis.

2 | METHODS

2.1 | Study design, dogs, and fecal sample collection

This period cross-sectional study (years 2019-2021) included fecal sample examinations of diarrheic and nondiarrheic kenneled dogs, all living in a single geographic area and exclusively fed commercial dry diets. The study was approved by the local institutional ethical committee (#HU-NER-2021-085-A). Dogs with known or estimated ages between <4 months or >5 years were excluded to minimize age-related fecal microbiome compositional changes.[27] Shelter dogs were included if they resided in the shelter for ≥3 months to allow sufficient historical data collection. Information including the dogs' general health and specific information regarding defecation frequency and fecal consistency (using the Purina 7-point fecal scoring chart descriptions)[28] was derived from kennel personnel. Dogs, nondiarrheic or diarrheic, were included only if deemed otherwise healthy, based on history, attitude, appetite, and physical examination (including body condition score of 4/9 to 5/9, as assessed by a single principal investigator). Dogs treated by any nutritional intervention or with any drug potentially affecting *Giardia* spp. or the fecal microbiome composition (eg, antibiotics, protein pump inhibitors) <3 months before fecal sample collection were excluded.[29]

A single fresh fecal sample from each dog was collected from individual kennel cages or during walks. Samples were tested for Gd infection within 1 hour of collection, using *Giardia* antigen dot ELISA

(ImmunoRun, Biogal Galed Labs, Galed, Israel).[30,31] Positive samples then were immediately frozen at −80°C for up to 2 years, pending microbiome analyses. Fecal consistency was determined visually upon sample collection by a single principal investigator, using the Purina 7-point fecal scoring chart. Diarrhea was defined based on scores ≥4.[28,32]

2.2 | 16S rRNA gene sequencing and analysis

Fecal sample DNA was extracted using the MagMax Microbiome Ultra Kit (Thermo fisher; Waltham, MA) following the manufacturer's instructions after a 2-minute bead beating step. The V4 region of the 16S rRNA gene was amplified using PCR, performed using 515F-barcoded and 806R-nonbarcoded primers.[33] Each PCR reaction consisted of 25 µL PrimeSTAR Max PCR mix (Takara Kusatsu, Shiga, Japan) and 2 µM of each primer, 17 µL of ultrapure water, and 4 µL DNA template. Thermal cycler conditions were as follows: 35 cycles with 10-second denaturation at 98°C, 5-second annealing at 55°C, and 5-second extension at 72°C, followed by 1-minute final elongation at 72°C. Amplicons then were purified using Kapa Pure magnetic beads (Roche; Basel, Switzerland) and quantified using the Quant-iT PicoGreen dsDNA Assay Kit (Invitrogen, Thermo Fisher; Waltham, MA). Equimolar amounts of PCR products then were pooled, and the pool was sequenced using the Illumina MiSeq platform (Genomic Center, Bar-Ilan University Azrieli Faculty of Medicine, Safed, Israel). Microbial communities then were analyzed (QIIME2 version 2022.02).[34] Single-end sequence reads were demultiplexed. Read errors were corrected by divisive amplicon denoising algorithm (DADA2).[34] Taxonomy was assigned against GreenGenes,[35] and a phylogenetic tree was generated. All analyses for the fecal samples were calculated based on a feature table, in samples containing ≥15 124 sequences. All samples were rarefied to this threshold.

Patterns of alpha (estimate_richness_function) diversity (Faith's phylogenetic diversity [PD])[36] and beta (distance function) diversity (weighted and unweighted UniFrac, Bray-Curtis and Jaccard) between the diarrheic and nondiarrheic groups were compared using Kruskal-Wallis and permutational multivariate analysis of variance (PERMANOVA), respectively, and separately by age (age ≤1 year or >1 year) and sex. Whereas alpha diversity is a measure of fecal microbial richness and evenness in samples, beta diversity is a measure of microbial community composition overlap among dogs. It is represented by principal coordinate analysis (PCoA), such that distances are calculated based on dissimilarities among communities, and then each dog's microbial community is plotted in the principal coordinate space. Points closer together represent microbiomes that are more similar to each other. When groups exhibit significantly different communities, this finding refers to differences in their mean placement in the principal coordinate space. Analysis of microbiome composition (ANCOM) at the phylum, genus and species levels was used to differentially identify abundant taxa between groups.[37]

2.3 | Microbial dysbiosis index

Fecal samples were shipped overnight on dry ice and confirmed to have arrived frozen to the Gastrointestinal Laboratory, Texas A&M University for analysis. The canine microbial dysbiosis index (DI) uses mathematical modeling based on specific fecal bacterial abundances measured in samples relative to a reference set from healthy dogs. This mathematical model provides a single numerical value, interpreted based on published reference intervals as follows: normal, <0; mild to moderate shift, 0 to 2; clinically relevant microbiota shift likely indicating dysbiosis, >2.[38] The Mann-Whitney test was used to compare the DI and its bacterial constituent quantities between diarrheic and nondiarrheic dogs.

2.4 | Additional statistical analyses

The Kolmogorov-Smirnov test was used to examine data distribution patterns. Qualitative and quantitative variables were compared between dog groups using the Fisher's exact and Mann-Whitney tests, respectively. A P value ≤.05 was considered significant. Statistical analyses were performed using a statistical software package (IBM SPSS 28.0.1.0, IBM, Armonk, NY).

3 | RESULTS

Fecal samples were collected from 131 dogs residing in municipal shelters (n = 3), private shelters (n = 2) and a commercial breeder kennel. *Giardia duodenalis* infection was diagnosed in 42 dogs (32%; diarrheic, 23 [55%]; nondiarrheic, 19 [45%]). All diarrheic dogs showed clinical signs compatible with small bowel diarrhea (ie, infrequent, large volumes of soft to unformed feces, with absence of blood or mucus), and with no systemic clinical signs (eg, dehydration). After DNA extraction, 38 samples (diarrheic, 21; nondiarrheic, 17) were available for 16S rRNA gene sequencing and 19 samples from each group were available for DI analysis. All dogs were fed commercially extruded dry diets, and were regularly dewormed upon entering the kennel and then every 3-6 months (Tables S1 and S2). No age, sex, or breed proportion differences were found between diarrheic and nondiarrheic dogs (Table 1).

3.1 | Fecal microbiome analyses

No difference in Faith's PD ($P = .54$) was found between diarrheic and nondiarrheic dogs (Figure 1A). When these groups were divided based on sex (males and females analyzed separately), diarrheic males had higher alpha diversity compared to diarrheic females (pairwise Kruskal-Wallis, $q = 0.01$; Figure 1B), but no differences were found between diarrheic and nondiarrheic dogs for either sex (pairwise Kruskal-Wallis, $q > 0.05$). Similarly, no age-specific effects were identified (pairwise Kruskal-Wallis, $q > 0.05$; Figure 1C).

TABLE 1 Signalment of 20 diarrheic and 18 nondiarrheic dogs naturally infected with *Giardia duodenalis*.

Variable	Diarrheic dogs (n = 20)	Nondiarrheic dogs (n = 18)	*P* value
Age (months)	Median, 18 (range, 4–28)	Median, 18 (range, 4-36)	.57
Sex	Females, 10 (50%) (intact, 3; neutered, 7)	Female, 4 (22%) (intact, 3; neutered, 1)	.07
	Male, 10 (50%) (intact, 2; neutered, 8)	Males, 14 (78%) (intact, 4; neutered, 10)	
Breed	Mixed breed (14; 70%), Belgian Malinois (n = 3) and Doberman pinscher, cavalier King Charles spaniel and dachshund (1 each)	Mixed breed (12; 67%), Shih Tzu (n = 3), and dachshund, Belgian Malinois and beagle (1 each)	–

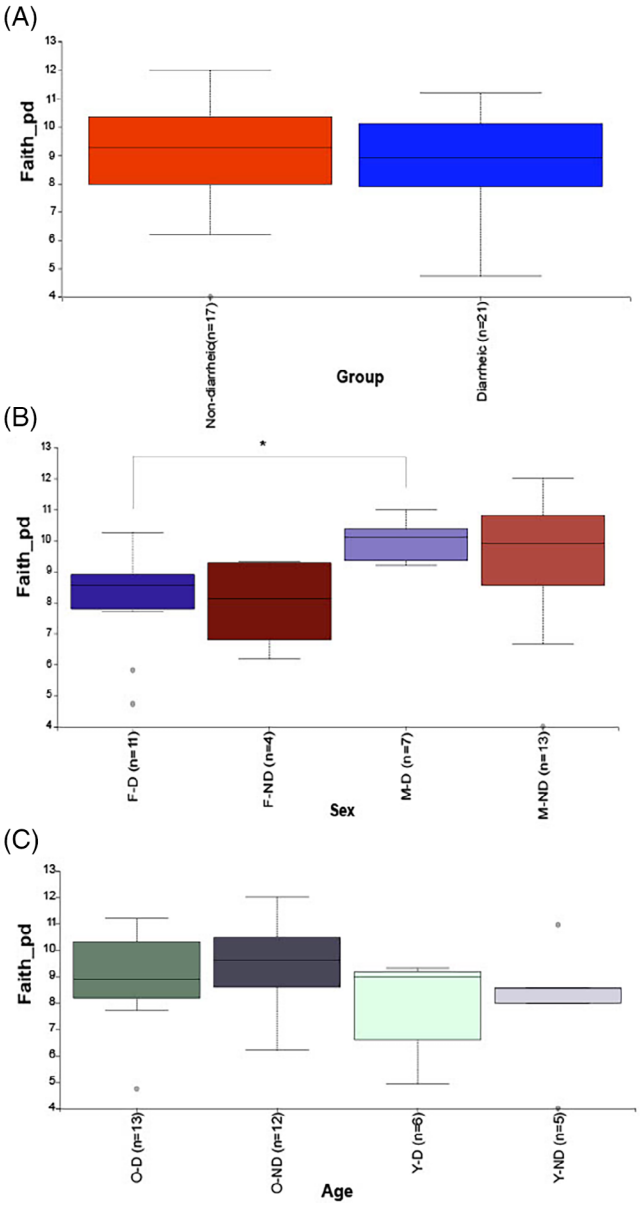

FIGURE 1 Fecal bacterial alpha diversity characterized using Faith's phylogenetic diversity ($P = .54$) in diarrheic and nondiarrheic groups (A), sex groups (B), and age groups (C) of 21 diarrheic and 17 nondiarrheic kenneled dogs naturally infected with *Giardia duodenalis*. F-D, diarrheic female; F-ND, nondiarrheic female; M-D, diarrheic male; M-ND, nondiarrheic male; O-D, diarrheic old; O-ND, nondiarrheic old; Y-D, diarrheic young; Y-ND, nondiarrheic young.

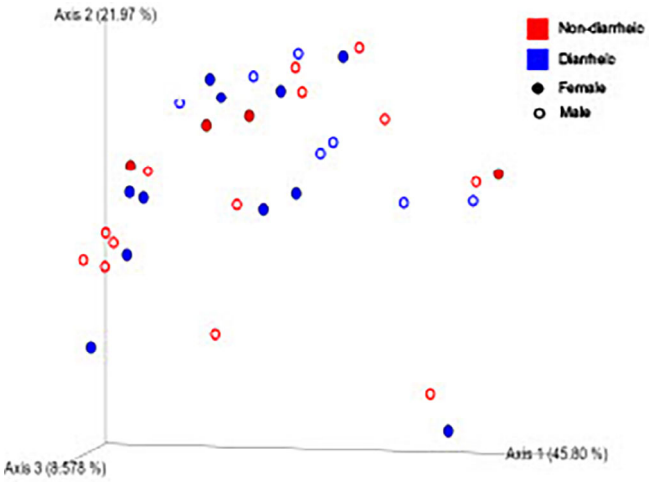

FIGURE 2 Fecal microbiota beta diversity based on weighted UniFrac distances for diarrheic and nondiarrheic males and females kenneled dogs naturally infected with *Giardia duodenalis*. Between group differences were compared with PERMANOVA ($q > 0.05$).

No beta diversity differences were detected between diarrheic and nondiarrheic dogs overall, based on weighted UniFrac distances ($P = .77$), or when examining sexes and age classes separately (Figures 2 and 3). Similar results were obtained using different metrics (unweighted UniFrac [$P = .44$], Bray-Curtis [$P = .4$] and Jaccard [$P = .49$]). Similarly, no differences were found in specific taxa abundances at the phylum, genus, and species levels between diarrheic and nondiarrheic dogs, when examined altogether. Bacteria of the genus *Clostridium* in general ($W = 19$), and particularly the species *Clostridium spiroforme* ($W = 33$), were more abundant in fecal samples of male diarrheic dogs compared to male nondiarrheic dogs (Figure 4); no differences were found among female dogs. Proteobacteria were more prevalent ($W = 1$) in fecal samples of diarrheic dogs <1 year of age compared to those of older diarrheic dogs, whereas *Verrucomicrobia* were more prevalent ($W = 1$) in the latter, compared to the former (Figure 5).

No difference ($P = .32$) was found between the overall fecal DI of diarrheic (median, −0.2; range, −4.3 to 4.5) and nondiarrheic (median, −1.0; range, −4.3 to 5.8) dogs nor in the quantities of its 7 specific bacterial taxa constituents (Figure 6).

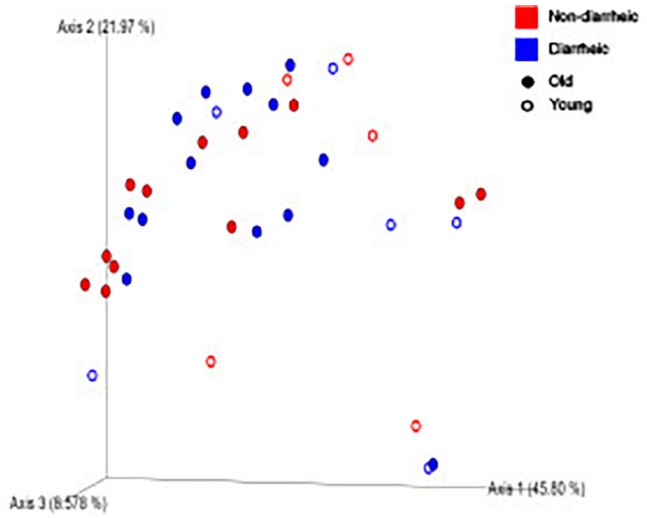

FIGURE 3 Fecal microbiota beta diversity based on weighted UniFrac distances for diarrheic and nondiarrheic dogs naturally infected with *Giardia duodenalis* and aged <1 year-young or ≥1 year-old. Between group differences were compared with PERMANOVA ($q > 0.05$).

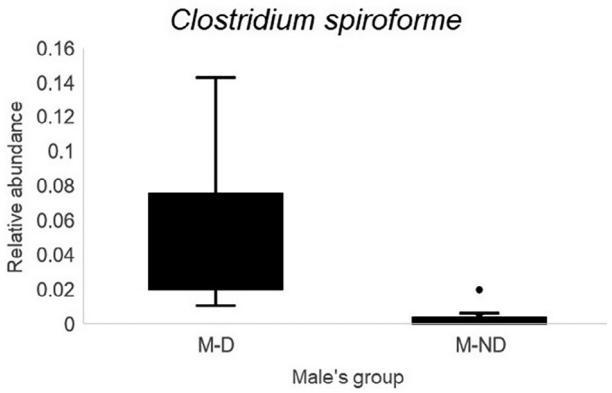

FIGURE 4 Analysis of composition of the fecal microbiome of 7 diarrheic and 13 nondiarrheic kenneled male dogs naturally infected with *Giardia duodenalis* shows significant group differences in prevalence of the genus *Clostridium* and the species *Clostridium spiroforme* ($W = 33$).

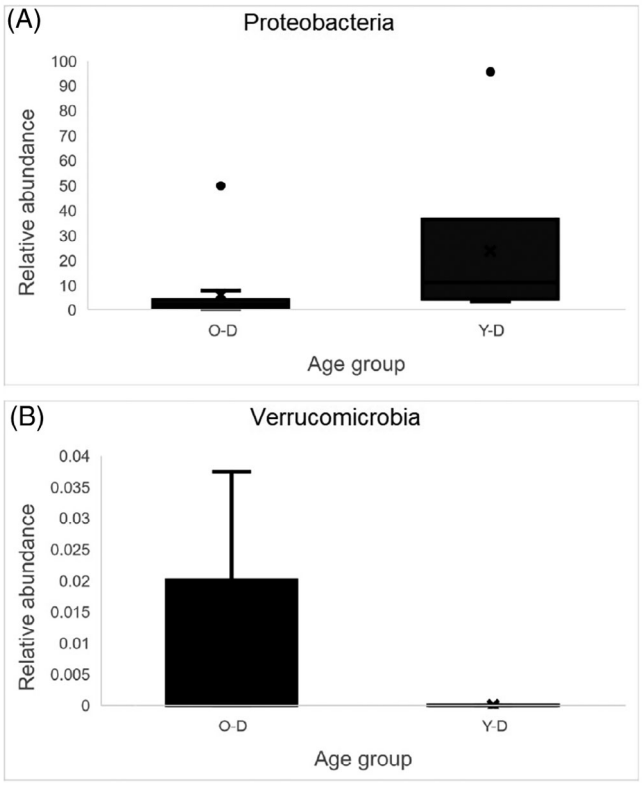

FIGURE 5 Analysis of composition of the fecal microbiome at the phylum level of 19 diarrheic dogs naturally infected with *Giardia duodenalis*, divided by age (≥1-year and <1-year) (O-D and Y-D, respectively). The prevalence of (A) *Proteobacteria* ($W = 1$) and (B) *Verrucomicrobia* ($W = 1$) differed significantly between groups. O-D: dogs >1 year old; n-13. Y-D: dogs <1 year old; n-6.

4 | DISCUSSION

We examined the associations between fecal microbiome characteristics and occurrence of clinical giardiasis (ie, diarrhea) in a cohort of young, otherwise healthy dogs infected with Gd, housed in similar kennel habitats, fed dry commercial diets, and not exposed to previous nutritional or pharmaceutical interventions. In contrast to previous studies in humans and animals, which reported significant intestinal microbiota composition shifts in Gd-infected hosts, that possibly contribute to the development and severity of clinical giardiasis,[3,21,22,25,26] our study suggests that in dogs naturally infected with Gd, fecal microbiome alterations do not differentiate diarrheic from nondiarrheic animals. The results suggest that other host- and Gd-related characteristics (eg, immunocompetence, parasite virulence[12]) are involved in the clinical expression and outcome of natural giardiasis in dogs, whereas the intestinal microbiome likely plays a more minor one.

Several factors might account for the differences in the results between our study and previous studies, regarding the presumed impact of microbial dysbiosis on the clinical signs of giardiasis. First, previous studies of Gd-associated microbiome changes used in vitro or experimental murine giardiasis models, which might not mimic the natural infection characteristics in the current cohort.[18,21,24,25] In support of this potential explanation for differences between study designs, a profound dysbiosis is noted in acutely-experimentally infected cats with *Tritrichomonas foetus* (*Tritrichomonas balgurni*), another intestinal protozoal parasite, but not in naturally-infected chronic carriers.[39] Furthermore, chronic *T. foetus* infection possibly conferred beneficial microbial changes, including increased abundance of short chain fatty acid producers important for colonocyte health (eg, *Megamonas* spp.) that could improve both parasite and host survival.[39] Previous studies reported similar Gd-induced microbial changes, including increased fecal microbial diversities and increased beneficial microbe abundances.[26] These changes might be sustained regardless of diarrheic status.

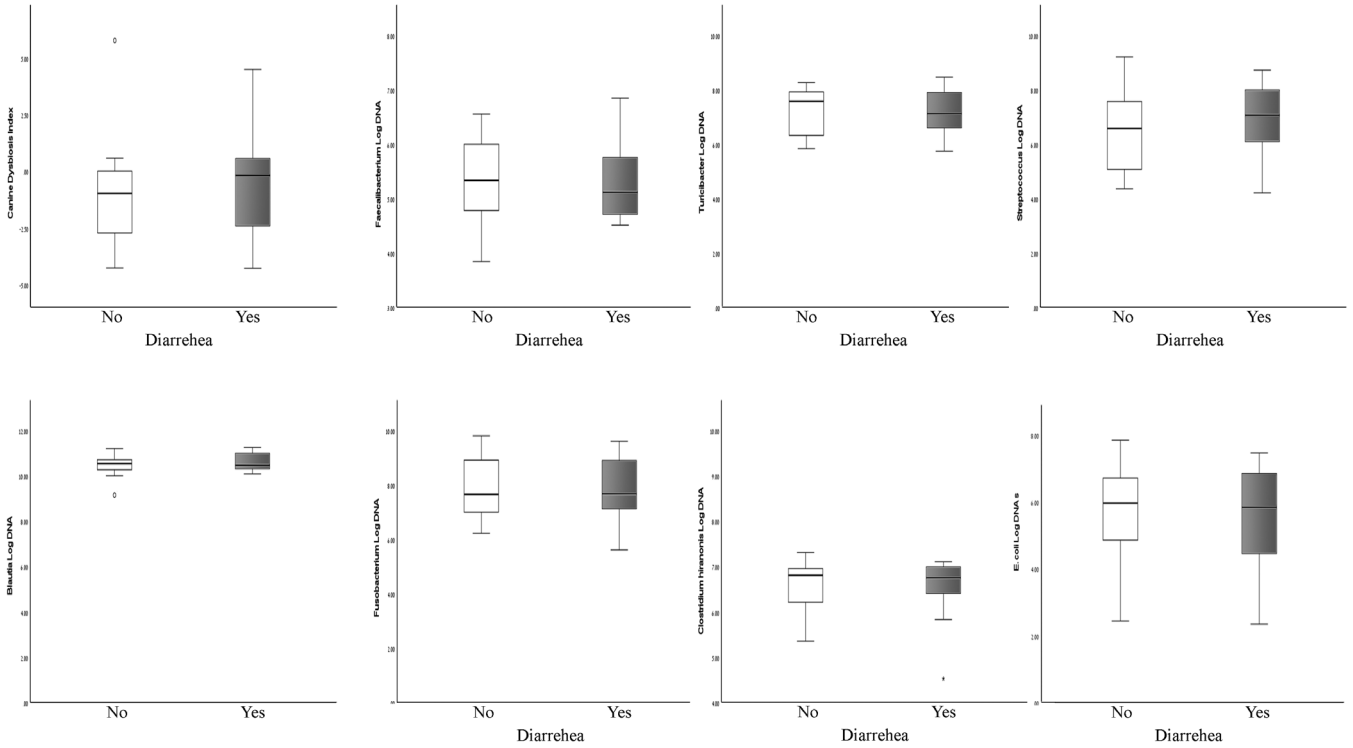

FIGURE 6 The fecal dysbiosis index (DI) and the relative abundance of 7 specific bacterial taxa in fecal samples of 21 diarrheic (group 1) and 17 nondiarrheic (group 2) kenneled dogs naturally infected with *Giardia duodenalis*. The DI is a quantitative PCR-based assay, used to assess the fecal microbiome in individual study participants.[38] The DI quantifies the fecal abundance of 7 bacterial taxa and total bacterial abundance. Normal DI (<0) indicates no overall diversity of the intestinal microbiota shifts. Mildly increased DI (0-2) suggests mild to moderate overall intestinal microbiota diversity shift. Markedly increased DI (>2) is consistent with a marked overall intestinal microbiota diversity shift. Data are shown as median, range, and quartiles. There was no difference ($P = .32$) in the DI of 17 diarrheic and 21 nondiarrheic dogs with giardiasis. The specific taxa, with their associated log DNA reference intervals in healthy dogs include *Faecalibacterium* (3.4-8.0), *Turicibacter* (4.6-8.1), *Blautia* (9.5-11.0), *Fusobacterium* (7.0-10.3), *Clostridium hiranonis* (5.1-7.1), *Streptococcus* (1.9-8.0), and *Escherichia coli* (0.9-8.0). In dysbiosis, the abundances of *Streptococcus* and *E. coli* increase, while those of all the other above-mentioned bacteria decrease.[38] There were no differences in the relative abundance of *Faecalibacterium* ($P = .64$), *Turicibacter* ($P = .93$), *Blautia* ($P = .58$), *Fusobacterium* ($P = .86$), *Clostridium hiranonis* ($P = 1.0$), *Streptococcus* ($P = .28$), and *Escherichia coli* ($P = .93$) between diarrheic and nondiarrheic Gd-infected dogs.

Second, previous studies of natural giardiasis in dogs included small cohorts, some of which were heterogeneous in age, diet, past drug treatment and habitat,[3,26,40] thereby introducing confounders potentially affecting fecal microbiome composition. Particularly, young age is the most reported factor determining the clinical severity of giardiasis in dogs, likely because of immune system immaturity and higher coinfection prevalence (eg, parvovirus infection in dogs).[1,4] In addition, age also affects the microbiome,[27,41] and thus its potential interplay with parasitic infections. Chronic subclinical giardiasis in puppies is associated with dysbiosis and increased fecal calprotectin concentration, suggestive of established chronic low-grade inflammation.[42] In support of our findings, these changes were not associated with clinical disease,[42] but might explain microbial feature differences among studies which include heterogenous age. In our study, *Proteobacteria* were more abundant in young diarrheic dogs compared to older (age >1 year) diarrheic dogs. *Proteobacteria* are dominant members in the gut of young mammals and play a key role in gut preparation for colonization by the strict anaerobes required for healthy gut function.[43] Thus, these differences among the different age classes in our study are to be expected. It therefore seems that comparing the microbiomes and their clinical association in dogs of variable ages with giardiasis might skew results. In our study, the group signalments were similar, but the main fecal microbial diversity parameters did not differ between diarrheic and nondiarrheic dogs, even when separately assessing age and sex groups, thereby eliminating these signalment differences as potential confounders. Interestingly, herein, *Clostridium* spp., and particularly the species *Clostridium spiroforme*, which are related to some enteric diseases in several animal species,[43] were significantly more abundant in diarrheic males compared to nondiarrheic males. Nevertheless, *Clostridium* spp. were not significantly more abundant in the entire group of diarrheic dogs as compared to the entire nondiarrheic group. Some studies have shown that sex-associated gut microbiota composition differences might play a role in sex differences noted in the development and course of various diseases.[44] Thus, we cannot exclude the association of *Clostridium* spp. with diarrheic status in male dogs with giardiasis.

The lack of associations between fecal microbial composition and presence or absence of diarrhea in Gd-infected dogs in our study is further supported by the similar DI of both dog groups. Additionally, the fecal DI of most dogs in our study was <0, suggestive of

normobiosis. Although dysbiosis and abnormal DIs might have been expected in dogs with diarrhea of any cause, profound dysbiosis was absent in the majority of diarrheic dogs in our study. Nonetheless, the presence of diarrhea does not necessarily indicate the presence of dysbiosis, as has been demonstrated by DI within reference range in various acute and chronic gastrointestinal diseases of dogs.[45,46] Furthermore, the current DIs might be reflective of previous results in animals and humans with giardiasis, showing increased fecal microbial richness (ie, higher alpha diversity), alongside microbial compositional patterns associated with health.[26] Therefore, the DI recorded in our study suggests that dogs with giardiasis do not suffer prominent dysbiosis, and that dysbiosis is not the primary contributor to development of diarrheal disease.

Our study had several limitations. First, the cohort size possibly negatively affected the power of the statistical analyses, particularly regarding female group proportions, Faith's PD, and DI of diarrheic dogs. Nevertheless, microbiome analyses showed no differences in sex between diarrheic and nondiarrheic dogs in our study, and weighted UniFrac beta-diversity results support our conclusions. Second, although investigating kenneled dogs decreases habitat heterogeneity, this design feature possibly was associated with inferior monitoring compared to privately owned dogs, because the clinical signs (eg, fecal score) were assessed based only on single visit. Nevertheless, historical information was gathered from experienced kennel personnel, who monitored the dogs several times daily, allowing the classification of diarrheic and nondiarrheic dogs. Thorough historical and physical examinations by kennel medical staff and by the investigators upon collection of fecal samples further minimized chances of erroneous health and fecal consistency assessments. Additionally, the diets were not standardized, which might have affected the clinical signs and fecal microbial composition. Nevertheless, all dogs were fed dry commercial diets exclusively, with identical ingredients and similar nutritional profiles, minimizing potential effects of diet types on the results.[40] Third, we did not include a control kenneled dog group uninfected by Gd. Nevertheless, examining the DI does allow assessing fecal microbiome deviations in comparison with the reference interval, which somewhat compensates for this limitation. In support of the current findings, a recent study comparing the fecal microbiome of asymptomatic Gd-infected dogs to healthy dogs, uninfected by Gd, also concluded that the presence of *Giardia* is associated with enrichment of protective bacterial taxa, which might limit host inflammation, and cause only minimal modification of gut microbial ecology. Thus, Gd-infected dogs do not sustain significant microbial compositional shifts compared to healthy dogs.[47] Fourth, Gd infection was diagnosed by fecal *Giardia* antigen detection, and not by molecular testing. While the diagnostic performances of ELISA for Gd antigen detection and PCR are similar,[1] the lack of PCR precluded identifying specific Gd assemblages. Nevertheless, presence of diarrhea has been reported to be unassociated with particular Gd assemblages in dogs.[48] Finally, in our study no investigation was conducted for presence of parasitic coinfections, precluding examining the potential impact of such coinfections on clinical signs and the fecal microbiome. Dogs with giardiasis show different microbial composition compared to healthy dogs, but no such difference is found between *Ancylostoma caninum*-positive and -negative dogs.[3] Exclusion of hookworm-positive dogs from the Gd-infected dog studies strengthens the differences between *Giardia*-positive and -negative dogs. This observation suggests that concurrent parasitic infections do somewhat affect the extent of giardiasis-associated intestinal microbial changes.[3] Nevertheless, *Ancylostoma caninum* infestation in dogs is absent in the region where our study was conducted, and extensive deworming protocols were implemented routinely in all kennels, decreasing the chances of clinically relevant helminthic coinfection and any impact of such coinfection on our results.

5 | CONCLUSIONS

Humans and dogs infected with Gd show intestinal microbial compositional changes compared to noninfected ones.[26] Nevertheless, our results suggest that fecal microbial composition does not differentiate symptomatic from nonsymptomatic dogs with giardiasis. Furthermore, profound dysbiosis, as reflected by the DI, was not a common feature in either group of Gd infected dogs in our study. Our findings suggest that other host and parasite characteristics play important roles in determining the severity of giardiasis in dogs and might affect the host-microbiome interplay in Gd infection. With their shared environments, similar omnivorous diets and intestinal structure, dogs are an ideal model system for translational gut microbiome research in humans.[26] Therefore, our findings might have implications across species. Because previous studies in mice do suggest that microbiome manipulation using pre- and postbiotics might benefit management of Gd-infected hosts,[49,50] our findings suggest that such an effect might not be translated into dogs with natural Gd infection, warranting dedicated in vivo studies of dogs and humans.

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CONFLICT OF INTEREST DECLARATION

Authors declare no conflict of interest.

OFF-LABEL ANTIMICROBIAL DECLARATION

Authors declare no off-label use of antimicrobials.

INSTITUTIONAL ANIMAL CARE AND USE COMMITTEE (IACUC) OR OTHER APPROVAL DECLARATION

Approved by the local institutional ethical committee (#HU-NER-2021-085-A). Voided fecal samples were collected with consent of kennels' management.

HUMAN ETHICS APPROVAL DECLARATION

Authors declare human ethics approval was not needed for this study.

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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