

Research Article

Effects of Epilepsy and Giardia intestinalis Infection on the Canine Gut Microbiome

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Abstract

The gut microbiome plays a critical role in maintaining host health by regulating immune function, metabolism, and physiological homeostasis. Variations in microbial composition have been associated with factors such as age, sex, genetics, geography, and disease status. Although the canine gut microbiome has received increasing attention, comprehensive investigations remain limited compared with human studies. This study analyzed publicly available canine gut microbiome datasets obtained from the National Center for Biotechnology Information (NCBI) Sequence Read Archive (SRA). A total of 982 samples (1.22 TB) collected from eight countries were included in the analysis.

Keywords: Gut microbiome, Biomarker, Epileptic, Giardia intestinalis, Fusobacterium; Species

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Abstract

Distance-based redundancy analysis (dbRDA) revealed significant associations between specific bacterial genera and disease conditions. Peptoclostridium was strongly associated with epilepsy, whereas Fusobacterium was predominantly associated with Giardia intestinalis infection. Network analysis based on Spearman rank correlation coefficients identified potential microbial interactions and candidate biomarkers. The findings demonstrate that canine gut microbiome composition differs according to disease status and suggest that specific microbial taxa may serve as biomarkers for epilepsy and giardiasis in dogs.

Introduction

The gut microbiome and host health are closely interconnected in mammals. Microbial communities contribute to immune regulation, nutrient metabolism, energy harvest, and the production of bioactive compounds. Disruptions in the balance between the host and gut microbiota, commonly referred to as dysbiosis, have been linked to numerous pathological conditions, including obesity, inflammatory bowel disease (IBD), and cancer. Alterations in microbial diversity and composition have also been associated with Crohn's disease, chronic inflammatory disorders, and respiratory diseases.

Recent studies have highlighted the importance of the gut–brain axis, demonstrating bidirectional communication between the gastrointestinal tract and the nervous system. Growing evidence suggests that changes in gut microbial communities may influence neurological disorders, including epilepsy. Similarly, gastrointestinal infections can significantly alter microbial composition, leading to disease-associated dysbiosis.

Despite increasing interest in companion animal microbiomes, large-scale analyses of canine gut microbial communities remain limited. Therefore, this study aimed to investigate the relationship between canine diseases and gut microbiome composition using publicly available sequencing datasets. Particular emphasis was placed on identifying microbial taxa associated with epilepsy and *Giardia intestinalis* infection as potential biomarkers.

Materials and methods

Data collection

Publicly available next-generation sequencing (NGS) datasets were obtained from the NCBI Sequence Read Archive (SRA). The initial dataset comprised approximately 1.22 TB of canine gut microbiome sequencing data. To ensure consistency and quality across studies, whole-genome sequencing data and reverse-end reads were excluded, resulting in approximately 62 GB of amplicon sequencing data for downstream analysis.

Data preprocessing

Sequence processing was performed using the DADA2 package in R. Low-quality reads, sequences lacking metadata, ambiguous sequences, and sequences shorter than 300 bp or longer than 500 bp were removed. Taxonomic classification was conducted using the SILVA database (version 138.1). Amplicon Sequence Variant (ASV) abundances were normalized to relative abundance prior to analysis.

Statistical and network analyses

Beta diversity was assessed at the ASV level using distance-based redundancy analysis (dbRDA) with Bray–Curtis dissimilarity measures. Statistical significance was evaluated using ANOSIM and ANOVA tests. Pairwise comparisons were performed using the Games–Howell test.

To identify disease-associated microbial interactions, network analysis was conducted at the genus level using Spearman rank correlation coefficients. Genera present in at least 5% of samples and exceeding 0.01% relative abundance were retained. Multiple testing correction was performed using the Benjamini–Hochberg method. Networks were constructed using correlation thresholds of $|\rho| > 0.6$ and $q < 0.01$ and visualized using Cytoscape.

Results

Gut microbiome community composition

Following quality filtering, 93,570,027 high-quality sequences were retained, resulting in 41,838 ASVs. These ASVs were classified into 55 phyla, 129 classes, 271 orders, 424 families, and 1,218 genera.

dbRDA analysis revealed significant differences in gut microbiome composition among disease groups. The genera *Peptoclostridium*, *Blautia*, and *Megamonas* were strongly associated with epilepsy, whereas *Fusobacterium*, *Bacteroides*, *Alloprevotella*, *Ligilactobacillus*, and *Prevotella_9* were associated with *Giardia intestinalis* infection.

At the genus level, *Peptoclostridium* was significantly enriched in epileptic dogs, while *Fusobacterium* dominated samples from dogs infected with *Giardia intestinalis*. In contrast, healthy dogs exhibited a more balanced and diverse microbial composition.

Network analysis

Network analysis demonstrated that microbial interactions were primarily dominated by members of the phylum Firmicutes, followed by Proteobacteria, Bacteroidota, and Actinobacteriota. Strong correlations were observed among disease-associated genera, with *Collinsella* exhibiting frequent associations with *Peptoclostridium*, *Blautia*, and *Megamonas*.

Further statistical analysis showed that *Fusobacterium* and *Ligilactobacillus* were significantly enriched in *Giardia intestinalis*

infections, whereas *Peptoclostridium* was highly abundant in epileptic dogs. Although *Bacteroides* did not reach statistical significance, it tended to be more prevalent in healthy animals.

Discussion

The gut–brain axis has emerged as an important area of research, particularly regarding neurological disorders such as epilepsy. Previous studies have reported reduced populations of bacteria involved in the production of gamma-aminobutyric acid (GABA) and short-chain fatty acids (SCFAs) in epileptic dogs. These metabolites play essential roles in neural regulation and intestinal health. Consistent with these findings, our analysis identified *Peptoclostridium* as a prominent microbial signature associated with epilepsy.

Giardia intestinalis is a widespread protozoan parasite that induces substantial alterations in the intestinal microbiota. Infection is often associated with an increase in opportunistic pathogens and pro-inflammatory bacteria, alongside reductions in beneficial microorganisms. In the present study, *Fusobacterium* exhibited the strongest association with *Giardia intestinalis* infection, accounting for a substantial proportion of the microbial community in affected dogs.

Interestingly, despite its high abundance, *Fusobacterium* displayed limited interactions within the microbial network, suggesting that its influence may occur independently of broader microbial associations. Conversely, *Ligilactobacillus*, a genus commonly regarded as beneficial, demonstrated extensive interactions, potentially reflecting a protective response against pathogen-associated dysbiosis.

Healthy dogs showed greater abundances of *Romboutsia* and *Turicibacter*, both of which have previously been associated with normal gut function and microbial stability. These findings further support the role of microbial balance in maintaining canine intestinal health.

Conclusion

This study identified several microbial taxa that may serve as biomarkers for disease-associated alterations in the canine gut microbiome. *Peptoclostridium* emerged as a potential biomarker for epilepsy, whereas *Fusobacterium* and *Ligilactobacillus* were strongly associated with *Giardia intestinalis* infection. The observed differences in microbial composition and interaction networks highlight the potential of gut microbiome profiling as a tool for disease detection and monitoring in dogs. Further experimental studies are needed to validate these candidate biomarkers and clarify their roles in disease pathogenesis.

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