Research & Reviews: Journal of Microbiology and Biotechnology e-ISSN: 2320-3528

Impact of Epilepsy and Giardia Intestinalis Infection on the Gut Microbiome in Canine Species

Chan-Yeong Park, Sung-Seok Lee, Hyun-Hee Seo, Dong Uk Ha, Ha-Da Jang, Hyun-Ah Yu, San Kim*

BRD, Bio Interaction Laboratory Room 1404, The First Tower, 10, Dongtan-daero21-gil, Hwaswong-si, Gyeonggi-do, South Korea

Research Article

Received: 09-May-2023, Manuscript No. JMB-23-98250; **Editor assigned:** 11-May-2023, PreQC No. JMB-23-98250(PQ); **Published:** 08-Jun-2023, DOI: 10.4172/2320-3528.12.2.005.

***For Correspondence:** San Kim, BRD, Bio Interaction Laboratory Room 1404, The First Tower, 10, Dongtan-daero21-gil, Hwaswong-si, Gyeonggi-do, South Korea

E-mail: sankim@brdc.biz

Citation: Kim S, et al. Impact of Epilepsy and Giardia Intestinalis Infection on the Gut Microbiome in Canine Species. RRJ Microbiol Biotechnol. 2023;12:005.

ABSTRACT

The role of the gut microbiome in host health is of growing interest, as it plays various roles in maintaining homeostasis. The composition of the microbiome has been shown to be associated with factors such as age, race, gender, geographical distribution, and genetic features. However, the full extent of the interaction between the gut microbiome and the host remains unknown. Compared to humans, there have been limited studies conducted on the gut microbiome of companion animals. In this study, we analyzed the canine gut microbiome using the National Center for Biotechnology Information (NCBI) SRA database. A total of 982 SRA data, comprising 1.22 TB, from 8 countries were included. Distance-based redundancy analysis (dbRDA) revealed a strong correlation between the presence of *Peptoclostridium* and epilepsy, and between the presence of *Fusobacterium* and Giardia intestinalis infection. Through network analysis using spearman rank-based correlation coefficient (ρ), we identified potential biomarkers by examining interactions within the genera lists. The results indicate that the composition of the canine gut microbiome varies with disease-specific microbes, suggesting that these species lists could serve as candidate biomarkers for epilepsy and Giardia intestinalis infection.

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

INTRODUCTION

The gut microbiome and host health are closely linked in mammals, and their interaction is essential for maintaining physiological functions such as immune regulation, production of bio-compounds, and energy harvesting. Imbalances in the microbiome-host interaction can lead to disorders. For example, the ratio of *Firmicutes* to *Bacteroidetes* has been correlated with obesity rates [1].

Changes in the composition of the gut microbiome, known as dysbiosis, characterized by a loss of species richness, have been associated with various physiological or pathological conditions such as Inflammatory Bowel Disease (IBD), obesity, and cancers [2,3]. Other studies have shown links between the gut microbiome and diseases such as crohn's disease, inflammatory intestinal disease, and chronic obstructive pulmonary disease [4-6]. Moreover, the gut microbiome is not only influenced by organs but also by the brain and the nervous system [7,8]. It has been found that the gut microbiome is significantly associated with various organs, including the brain.

In this study, we aimed to obtain meaningful insights from initial research on animal gut microbiomes using the NCBI database. Our objective was to investigate the correlation between diseases and the gut microbiome and identify genera that may have an impact on specific diseases. Subsequently, we considered these genera as potential biomarker candidates.

MATERIALS AND METHODS

Data collection

We utilized publicly available Next-Generation Sequencing (NGS) data obtained from the National Center for Biotechnology Information (NCBI), specifically from the uploaded SRA projects, to analyze the composition of the canine gut microbiome. The total size of the downloaded canine gut microbiome sequence data from the SRA project was 1.22 TB. To ensure the analysis was conducted under consistent and high-quality conditions across all samples worldwide, we focused on 62 GB of amplicon sequences after sorting out Whole-Genome Sequencing (WGS) sequences and reverse-end sequences.

Data pre-processing

The raw data underwent pre-processing using the "dada2" R package [9] with the single-end forward sequences obtained from the NCBI amplicon sequences. The pre-processing steps involved excluding low-quality sequences and those with unavailable metadata. Additionally, sequences containing ambiguous characters and those shorter than 300 bp or longer than 500 bp were removed. To align and classify the sequences, we utilized the latest Silva database (version 138.1, Jan. 2023) [10]. The abundance of Amplicon Sequence Variants (ASVs) was normalized to relative abundance for subsequent analysis.

Microbial composition with statistical analysis

To estimate the clustering of gut microbiome based on metadata, beta-diversity analysis was conducted at the ASV level. This analysis utilized the distance-based redundancy analysis (dbRDA) with the 'capscale' algorithm from the "vegan" package in R. The results were visualized using the "ggplot2" package [11,12]. In this analysis, bray distance permutations were performed 9999 times [13,14]. Differentiation was assessed using statistical analysis of similarities (ANOSIM) and analysis of variance (ANOVA<0.01) from the "vegan" package in R [13,15]. Pairwise tests were conducted using the games-howell test, and visualization of the results was performed with the "ggstatsplot" package in R [16].

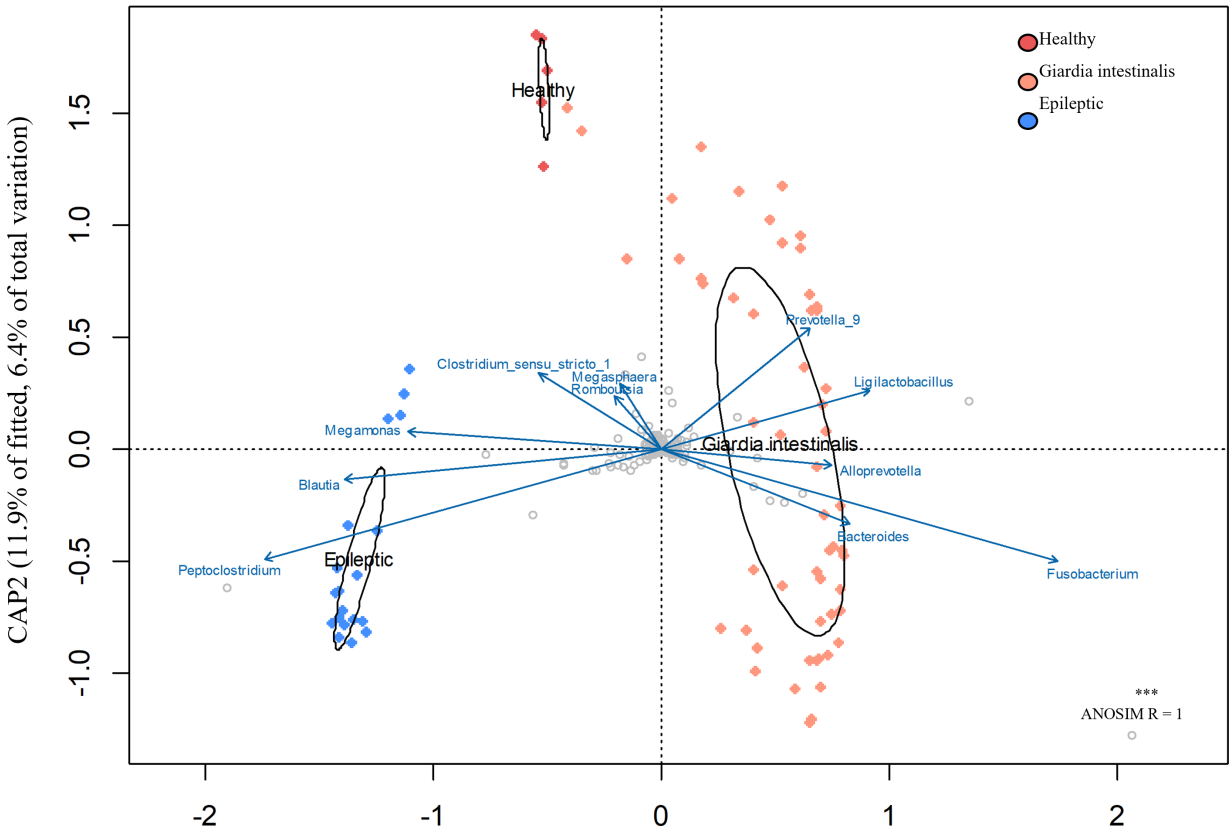
To explore the relationships between disease and the gut microbiome, network analysis was conducted at the genus level based on the spearman rank-based correlation coefficient (ρ). This analysis aimed to identify potential interacting candidate biomarkers [17]. Only genera that met the following thresholds were considered: Detected in at least 5% of the samples and relative abundance of less than 0.01% [18]. To address multiple testing errors, p-values were adjusted using the Benjamin-Hochberg method, and the network was constructed with the following thresholds: ρ (absolute value)>0.6 and q-value<0.01 [19]. Network visualization was performed using cytoscape tools [20].

RESULTS

Gut microbiome community composition

After the processing steps, a total of 93,570,027 high-quality sequences were retained, resulting in the identification of 41,838 Amplicon Sequence Variants (ASVs). These ASVs were classified into 55 phyla, 129 classes, 271 orders, 424 families, and 1,218 genera. According to the distance-based redundancy analysis (dbRDA), there were significant differences in gut microbiome compositions at the ASV level between disease groups (ANOSIM R=1, p-value<0.001) (Figure 1). Specifically, the genera *Peptoclostridium*, *Blautia*, and *Megamonas* were correlated with the epileptic group, while *Fusobacterium*, *Bacteroides*, *Alloprevotella*, *Ligilactobacillus*, and *Prevotella*_9 were correlated with the Giardia intestinalis group (Figure 1).

Figure 1. Distance-based redundancy analysis (dbRDA) between disease groups and healthy groups. **Note:** Healthy; Giardia intestinalis; Epileptic.



Furthermore, at the genus level, *Peptoclostridium* was found to be highly abundant in the epileptic group, while *Fusobacterium* dominated in the Giardia intestinalis group, both showing statistically significant differentiation between disease groups (ANOSIM R=0.6376, p-value<0.001) (Figure 2). These bacterial genera were considered as candidate biomarkers for the respective diseases, detectable through gut microbiome analysis. In contrast, the gut microbiome composition of the healthy group exhibited similar and balanced compositions among genera (Figure 2). These composition patterns were consistent with the evenness observed in the alpha diversity analysis (Figure 3).

Figure 2. Relative abundance of top 20 genus between the disease groups and healthy group. **Note**: Others; *Lachnoclostridium*; *Terrisporobacter*; *Collinsella*; *Sutterella*; *Anaerobiospirillum*; *Catenibacterium*; *Faecalibacterium*; *Escherichia-Shigella*; *Clostridium* sensu stricto 1; *Streptococcus*; [*Ruminococcus*] gnavus group; *Cetobacterium*; *Alloprevotella*; Romboutsia; *Megamonas*; *Prevotella_9*; *Bacteroides*; *Blautia*; *Peptoclostridium*; *Fusobacterium*.

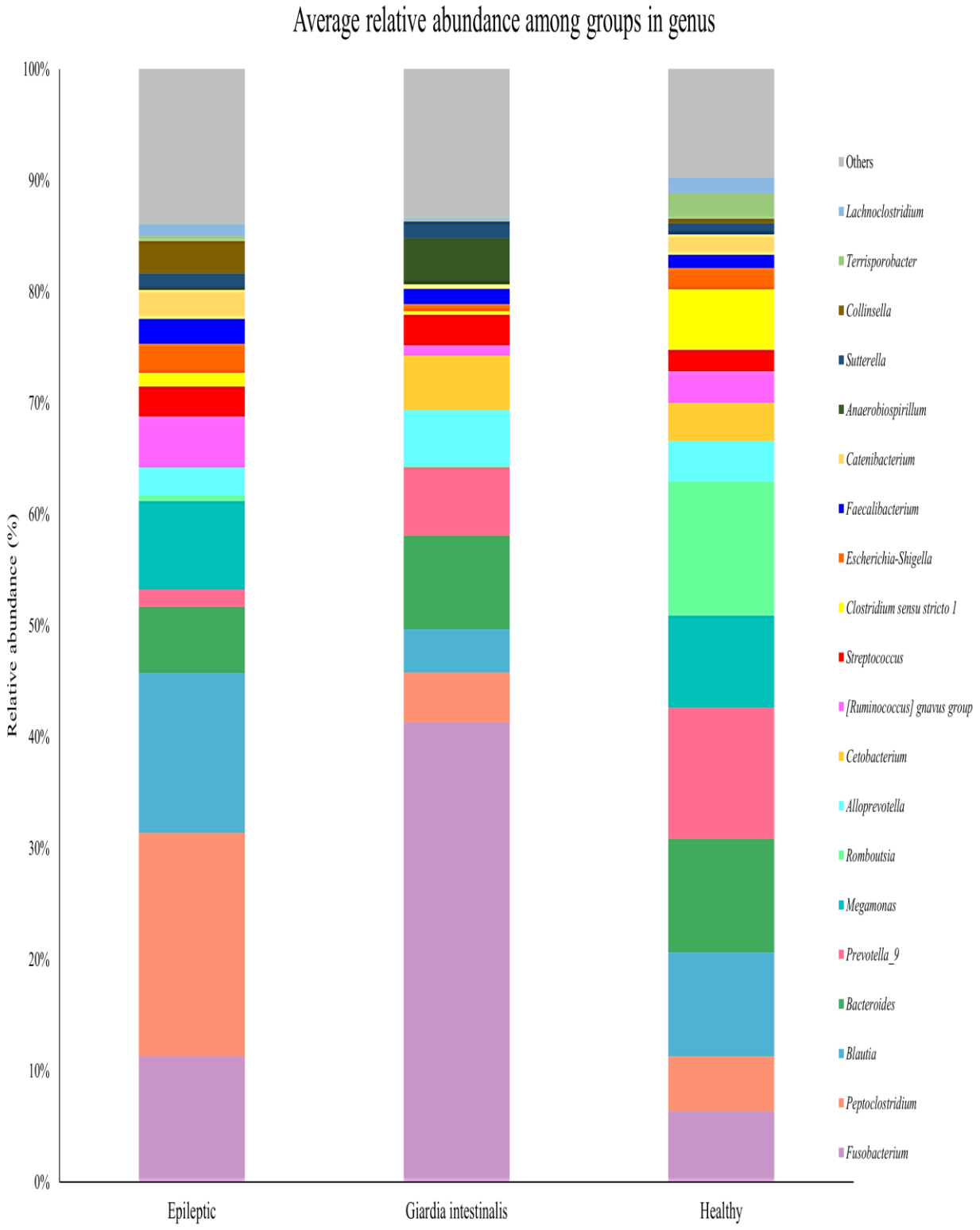
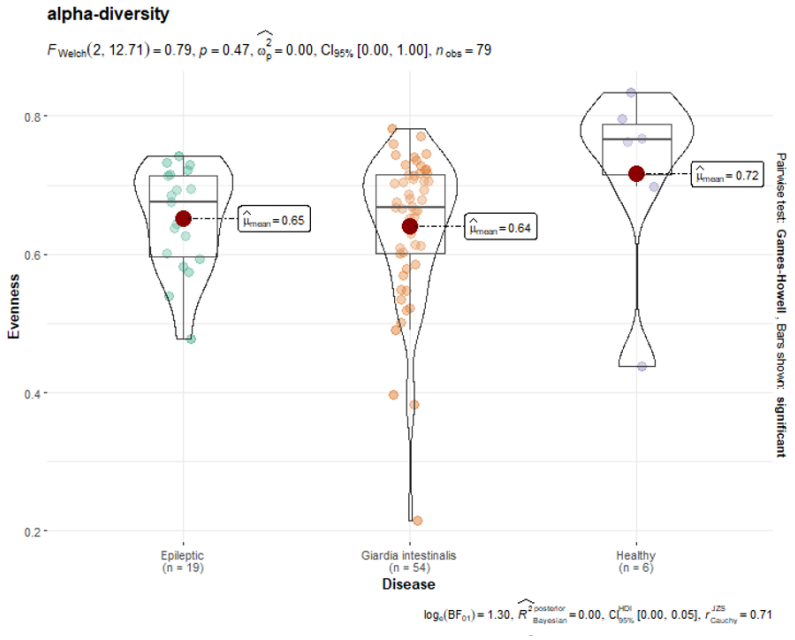


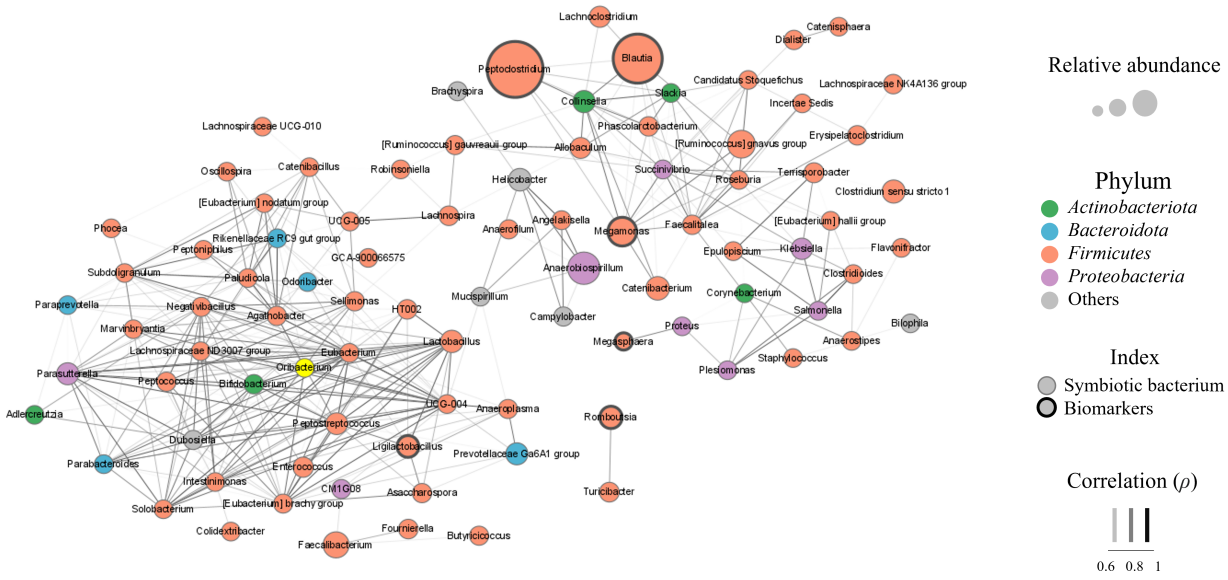
Figure 3. Evenness of alpha-diversity analysis with pairwise test. **Note:** ● Mean values.



Biomarker analysis

To explore the interacting relationships among the candidate biomarkers, a network analysis was performed using spearman rank-based analysis. Interestingly, the network was primarily dominated by *Firmicutes* at the phylum level, followed by Proteobacteria, *Bacteroidota*, and *Actinobacteriota* (Figure 4). Each candidate biomarker exhibited strong correlations within the network (Figure 4). Notably, *Collinsella* showed the strongest and most frequent correlations with *Peptoclostridium*, *Blautia*, and *Megamonas* (Figure 4).

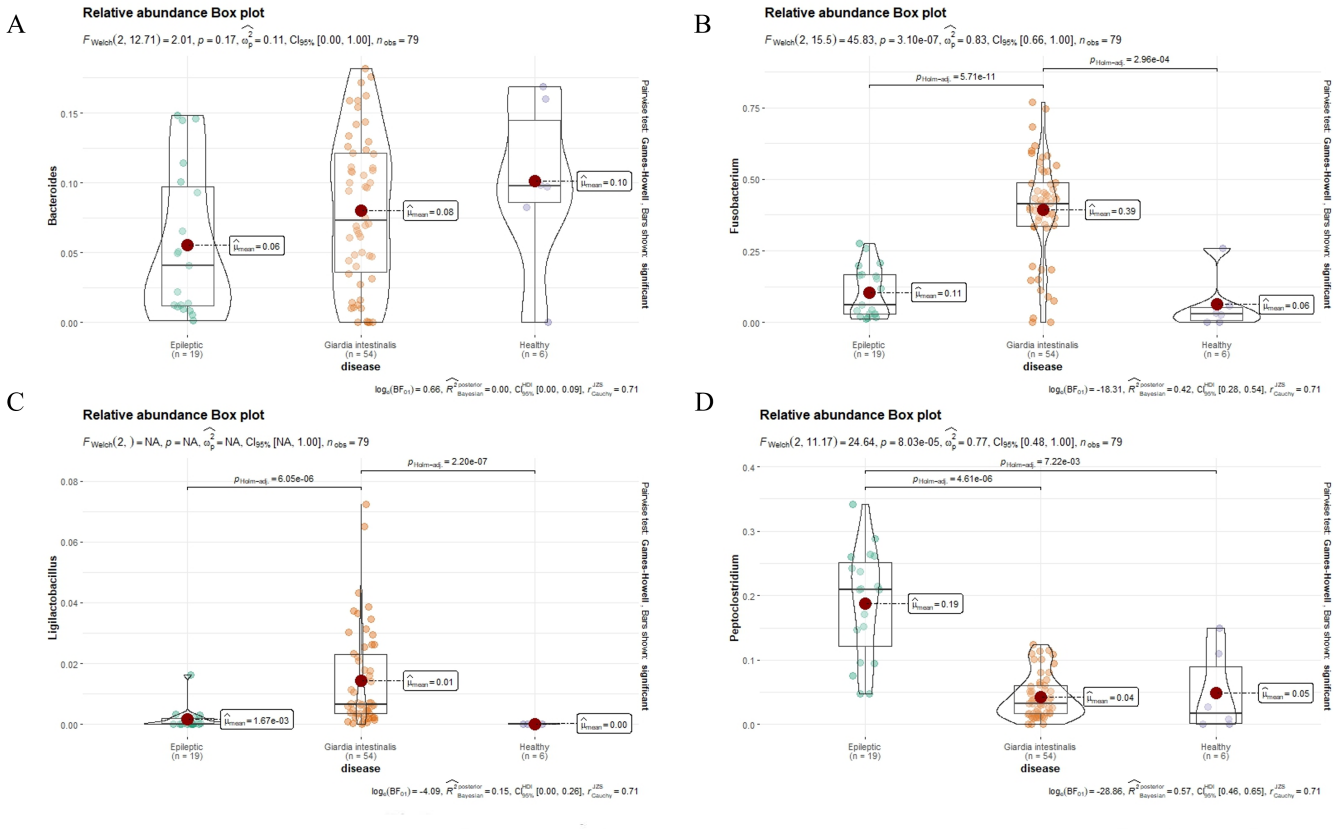
Figure 4. Network analysis based on spearmans rank-based correlation coefficient. **Note:** ● Relative abundance; ● Actinobacteriota; ● Bacteroidota; ● Firmicutes; ● Proteobacteria; ● Others; ● Symbiotic bacterium; ○ Biomarkers; ■ 0.6; ■ 0.8; ■ 1.



To further investigate the biomarker candidates, more specific information was obtained from box plots with statistical analysis (Figures 5A-5D). It was observed that *Fusobacterium* and *Ligilactobacillus* were highly abundant in cases of Giardia intestinalis disease, while *Peptoclostridium* was the dominant genus in cases of epileptic disease. Although the results for *Bacteroides* did not show statistical significance, there were trends suggesting that this genus predominantly occupied a niche in healthy conditions (Figures 5A-5D).

Figure 5. Box plots with pairwise test of statistical analysis in most significant genus at each category between the disease groups and healthy group for A) Bacteroides, B) Fusobacterium, C) Ligilactobacillus and D) Peptoclostridium.

Note: ● Mean values.



DISCUSSION

In recent years, there has been significant research on the brain-gut axis, exploring the relationship between the gut microbiome and neurodegenerative diseases in the canine model [7]. Epilepsy, being an encephalopathy, is caused by factors such as orexins, oxidative stress, and cerebral toxocariasis, among others, although the exact reasons remain unknown [21-23]. Consistent with previous studies, it has been observed that bacteria involved in the production of γ -aminobutyric acid (GABA) and Short-Chain Fatty Acids (SCFAs) are significantly reduced in epileptic beagles [24]. Additionally, the family Prevotellaceae Ga6A1 group has been associated with a reduced risk of brain disease [24]. These findings clearly indicate the influence of gut microbiomes on brain diseases [24].

Giardia intestinalis is a flagellated protozoan that causes giardiasis, a parasitic infection prevalent in humans and mammals worldwide [25]. When infected with Giardia intestinalis, the gut microbiota undergoes structural changes, becoming enriched with facultative anaerobic, mucus-degrading, pro-inflammatory species, and opportunistic pathogens [25]. Additionally, this infection has been shown to reduce the presence of *Lactobacillus* at specific time points in canines [25].

In our study, we observed that the pathogen *Fusobacterium* exhibited the strongest correlation with Giardia intestinalis infection, followed by the *Prevotella*, including *Ligilactobacillus* and *Bacteroides* (Figure 1). Notably, *Prevotella*_9 and *Ligilactobacillus* appeared to mainly influence the upper portion of CAP2, while *Alloprevotella*, *Bacteroides*, and *Fusobacterium* affected the lower portion (Figure 1). This suggests that the clustering of Giardia intestinalis infection extends across a wide range in the y-axis, and the effectiveness of different genera may vary in different parts of the clustering (Figure 1).

The Giardia intestinalis group exhibited a high dominance of *Fusobacterium*, accounting for over 40% of the genus composition in the gut (Figure 2). However, in the network analysis, *Fusobacterium* was not detected, indicating that it may impact Giardia intestinalis disease without any significant interactions with other species (Figure 4). On the other hand, *Ligilactobacillus* displayed complex interactions in the context of Giardia intestinalis disease, suggesting that the interacting genera may attempt to protect against *Fusobacterium* in the presence of Giardia intestinalis infection, as *Ligilactobacillus* is well-known as a probiotic in the gut system (Figure 4).

It has been observed that *Romboutsia* in the gut microbiome is generally associated with healthy adult canines [26]. Consistent with this, the healthy groups in our study exhibited a higher relative abundance of *Romboutsia* compared to the disease groups (Figure 2). Additionally, *Turicibacter* was identified as an interacting bacterium with *Romboutsia* and showed a higher relative abundance in the healthy groups compared to the disease groups (Figures 2 and 4). These interaction relationships and correlations highlight the need for further investigation to understand how these candidate biomarkers influence disease in the gut microbiome and the underlying mechanisms involved.

CONCLUSION

In our study, we identified several biomarker candidates for the diseases epileptic and Giardia intestinalis. After statistical analysis, we were able to narrow down the biomarker candidates with significant results (Figure 5). *Fusobacterium* and *Ligilactobacillus* emerged as potential biomarker candidates for Giardia intestinalis disease, while *Peptoclostridium* could serve as a biomarker candidate for epileptic disease (Figure 5).

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

In the current study, there was no need for ethics approval and written consent since this study did not implement any live material.

CONSENT FOR PUBLICATION

All authors agreed for the publication of the current study.

AVAILABILITY OF DATA AND MATERIAL

The datasets generated during and/or analyzed during the current study are available in the [NCBI] repository [https://www.ncbi.nlm.nih.gov/Traces/study/?query_key=1&WebEnv=MCID_63fc3ee01432dc33cb454230&o=acc_s%3Aa].

COMPETING INTERESTS

We declare that the authors have no competing interests as defined by BMC, or other interests that might be perceived to influence the results and/or discussion reported in this paper.

FUNDING

We are supported from BRD Corp., Bio Interaction Laboratory Room 1404, The First Tower, 10, Dongtan-daero21-gil, Hwaswong-si, Gyeonggi-do, South Korea.

AUTHORS CONTRIBUTIONS

CY Park gathered open source data and analyzed with interpretation of the data regarding the disease and gut microbiome. CY Park wrote the main manuscript texts. SS Lee discussed all results and gave ideas for the analysis methods and title. S Kim* performed discussion of results and ideas of the analysis methods. HH Seo, DU Ha, HD Jang, and HA Yu revised and discussed the manuscript.

ACKNOWLEDGEMENTS

Authors appreciated with Jung-Hyun Park for assistance of the sample data gathering and Antonio Kim for the general laboratory management.

REFERENCES

1. Turnbaugh PJ, et al. An obesity-associated gut microbiome with increased capacity for energy harvest. Nature. 2006;444:1027-1031.
2. Zhang YJ, et al. Impacts of gut bacteria on human health and diseases. Int J Mol Sci. 2015;16:7493-7519.
3. Shreiner AB, et al. The gut microbiome in health and in disease. Curr Opin Gastroenterol. 2015;31:69-75.
4. Valdes AM, et al. Role of the gut microbiota in nutrition and health. BMJ. 2018;13:361.
5. Seksik P, et al. Alterations of the dominant faecal bacterial groups in patients with crohns disease of the colon. Gut. 2003;52:237-242.
6. Bowerman KL, et al. Disease-associated gut microbiome and metabolome changes in patients with chronic obstructive pulmonary disease. Nat Commun. 2020;11:5886.
7. Ambrosini YM, et al. The gut-brain axis in neurodegenerative diseases and relevance of the canine model: A review. Front Aging Neurosci. 2019;11:130.
8. Grenham S, et al. Brain-gut-microbe communication in health and disease. Front Physiol. 2011;2:94.
9. Callahan BJ, et al. DADA2: High-resolution sample inference from illumina amplicon data. Nat Methods. 2016;13:581-583.
10. Yilmaz P, et al. The SILVA and “all-species living tree project (LTP)” taxonomic frameworks. Nucleic Acids Res. 2014;42:643-648.
11. Legendre P, et al. Distance-based redundancy analysis: Testing multispecies responses in multifactorial ecological experiments. Ecol Monogr. 1999;69:1-24.
12. Wickham H, et al. Package ‘ggplot2’. An implementation of the grammar of graphics. 2014.

13. Anderson MJ, et al. PERMANOVA, ANOSIM, and the Mantel test in the face of heterogeneous dispersions: What null hypothesis are you testing?. Ecol Monogr. 2013;83:557-574.

14. Oksanen J, et al. The vegan package. Community Ecology Package. 2007;10:1-190.

15. St L, et al. Analysis of variance (ANOVA). Chemometr Intell Lab Syst. 1989;6:259-272.

16. Patil I. Visualizations with statistical details: The 'ggstatsplot' approach. J Open Source Softw. 2021;6:3167.

17. Zar JH. Significance testing of the spearman rank correlation coefficient. J Am Stat Assoc. 1972;67:578-580.

18. Chun SJ, et al. Characterization of distinct cyanoHABs-related modules in microbial recurrent association network. Front Microbiol. 2019;10:1637.

19. Storey JD. The positive false discovery rate: A bayesian interpretation and the q-value. Ann Stat. 2003;31:2013-2035.

20. Shannon P, et al. Cytoscape: A software environment for integrated models of biomolecular interaction networks. Genome Res. 2003;13:2498-2504.

21. Erken HA, et al. Orexins cause epileptic activity. Peptides. 2012;37:161-164.

22. Patel M. Mitochondrial dysfunction and oxidative stress: Cause and consequence of epileptic seizures. Free Radic Biol Med. 2004;37:1951-1962.

23. Bachli H, et al. Cerebral toxocariasis: A possible cause of epileptic seizure in children. Childs Nerv Syst. 2004;20:468-472.

24. Garcia-Belenguer S, et al. Gut microbiota in canine idiopathic epilepsy: Effects of disease and treatment. Animals. 2021;11:3121.

25. Boucard AS, et al. Age and Giardia intestinalis infection impact canine gut microbiota. Microorganisms. 2021;9:1862.

26. Nybroe S, et al. Alterations in healthy adult canine faecal microbiome and selected metabolites as a result of feeding a commercial complete synbiotic diet with *Enterococcus faecium* NCIMB 10415. Animals. 2022;13:144.