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Research Article
New Insights into Bacterial Communities in Cat Faeces Containing Roundworm Eggs

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Abstract

Background and Objective: Pathogenic microorganisms such as bacteria and parasites cause disease and ill health in domestic pets, including cats. However, few studies have examined the composition of the bacterial community in cat faeces containing helminth eggs. To bridge this knowledge gap, study aimed to investigate bacterial microbiota in cat faeces containing nematode eggs. **Materials and Methods:** Bacterial analysis was performed on cat faeces targeting the V3-V4 region of 16S rDNA using next-generation sequencing technology. Samples of cat faeces that contained roundworm eggs were pooled as Group 1, whereas faeces without roundworm eggs placed in Group 2. Results were analysed using R (v3.3.1), SPSS (Chicago, IL, USA) and LEfSe (v1.0) for visualization, statistical testing and differential taxa abundance analysis, respectively ($p<0.05$). **Results:** Groups 1 and 2 shared 306 OTUs (Operational Taxonomic Units). In different OTUs, Group 1 contained 324 and Group 2 was 185 OTUs. At the phylum level, Bacteroidota dominated the community composition of Groups 1 and 2. Firmicutes were abundant in both groups. Campylobacterota were more abundant in Group 1 than in Group 2. However, Fusobacteriota was more predominant in Group 2 than in Group 1. At the genus level, *Helicobacter* was higher in Group 1 than in Group 2. In contrast, *Bacteroides*, *Fusobacterium* and *Collinsella* were more abundant in Group 2 than in Group 1. **Conclusion:** These results provide the first evidence of the bacterial community and bacterial core in cat faeces containing roundworm eggs.

Key words: Faecal bacteria, nematode, feline, 16S rDNA, animal health

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Domestic cats are among the most popular pet animals globally and the relationship between humans and cats is defined by utilities such as companionship and emotional comfort[1,2]. However, pathogenic organisms (primarily bacteria, parasites, viruses and fungi) are the leading causes of illness in domestic cats and zoonotic risks to humans[3-5]. Common pathogens found in cat faeces can transmit zoonotic diseases[5,6]. The diversity of feline pathogens has been studied to understand host-pathogen interactions and important components of organisms in the health of cats[3,7-9]. For instance, Bai *et al*.[10] showed that the gut microbiota in faecal samples from healthy cats contained *Prevotella*, *Providencia* and *Sutterella*, whereas samples from cats with acute diarrhoea showed an abundance of *Bacteroidota* and *Prevotella*. Tal *et al*.[11] also reported that bacterial communities in Firmicutes were enriched in faecal samples of obese cats compared to lean cats. Furthermore, Sung *et al*.[12] demonstrated that the faecal abundances of *Escherichia coli* and *Streptococcus* were higher in cats with chronic enteropathies than in healthy cats. Remesar *et al*.[13] found that cats co-infected with *Toxocara cati* showed a higher prevalence of Metastrongylidae. Joachim *et al*.[6] reported that the most common roundworm found in cat faeces was *T. cati* and the zoonotic bacteria were *Campylobacter jejuni* and *Yersinia enterocolitica*. Moreover, Ursache *et al*.[14] revealed the identification of intestinal parasites such as *T. cati* with other parasitic enteropathogens. From the above studies, however, little is known about how the presence of helminth eggs affects the microbiota composition in feline faeces.

Next-generation sequencing (NGS), also known as massively parallel or deep sequencing, enables the simultaneous sequencing of millions of DNA fragments and increases data output and operational efficiency[15,16]. For example, Gebremariam *et al*.[17] successfully assessed the faecal microbiota composition of cats infected orally with feline infectious peritonitis virus and treated them with antiviral drugs based on 16S rRNA sequences via NGS. Kitson *et al*.[18] provided and described important data on bacterial communities and structures in the blood of febrile and healthy cats using 16S rRNA-based NGS. This study aimed to examine the bacterial microbiota in cat faeces containing roundworm eggs. This study is expected to offer important information on the microbial diversity of felines.

MATERIALS AND METHODS

Study area and duration: Samples of cat faeces were collected from local animal hospitals and veterinary clinics in the suburbs of Bangkok during November, 2024 to March, 2025. The experiments were conducted at the Department of Environment and Natural Resources, Faculty of Environmental Culture and Eco-tourism, Srinakharinwirot University, Thailand.

Sample collection and grouping: To set similar conditions, such as the same sex and adult age, all faecal samples from cats were kept in absolute ethanol at low temperature (4°C). Some faecal materials were separated to identify parasite eggs. Faecal aliquots were used to identify parasitic eggs according to the methods described by Garcia *et al*.[19]. In this study, we only collected faecal samples with nematode eggs. Samples that were found to contain nematode eggs were used as Group 1 (cats infected with roundworms) and samples without roundworm eggs were selected as Group 2 (control group or healthy cats). For biological replication, faecal samples were separated from three independent cats in each group and subjected to repeated statistical analyses.

DNA extraction and quantification: Total genomic DNA was extracted from cat faecal samples using the QIAamp Fast DNA Stool Mini Kit (Qiagen, Hilden, Germany) according to the manufacturer's instructions. Accurate DNA quantification was assessed with a Qubit® dsDNA high-sensitivity assay kit (Qubit, Hilden, Germany).

Library preparation and sequencing: Library sequencing was performed using a MetaVX Library Preparation Kit supplied by GENEWIZ (South Plainfield, NJ, USA). Amplicons were generated from DNA samples covering the hypervariable regions of the bacterial 16S rRNA gene (V3-V4). The PCR conditions for amplifying the V3-V4 region with universal primers were as follows: Pre-denaturation at 94°C for 3 min, 26 cycles (denaturation at 94°C for 30 sec, annealing at 57°C for 40 sec and extension at 72°C for 60 sec) and a final extension at 72°C for 5 min. The NGS was performed using an Illumina MiSeq/NovaSeq platform (Illumina, San Diego, CA, USA and GENEWIZ, South Plainfield, NJ, USA).

OTU clustering and taxonomic assignment: For operational taxonomic unit (OTU) clustering, sequence similarity was established at a 97% identity threshold using Vsearch (v1.9.6) and the 16S rRNA database described by Silva (138). The Ribosomal Database Program classifier, using the Bayesian algorithm, was used to create representative OTU sequences at the taxonomic level.

Data analysis: Results, such as Venn diagrams and stacked bar plots, were obtained using R software (3.3.1)[20]. Diversity indices were calculated and significant differences ($p<0.05$) were analysed by the Mann-Whitney U test using the Statistical Package for Social Sciences (SPSS) (Chicago, IL, USA)[21]. Finally, linear discriminant analysis (LDA) effect size (LEfSe) was used to test for significantly different abundances of bacterial taxa using LEfSe (1.0)[22].

RESULTS

In the identification of bacteria, 324 OTUs were found only in the faecal samples of cats infected with roundworms (Group 1). Conversely, 185 OTUs were specific to faecal samples without nematode eggs (Group 2). In addition, 306 bacterial OTUs were shared between the faecal samples of cats in Groups 1 and 2 (Fig. 1a). A total of 10 phyla with 109 genera were detected. At the phylum level, the most abundant phylum in Groups 1 and 2 was *Bacteroidota*. Furthermore, these groups were characterised by the predominance of Firmicutes. Proteobacteria were also abundant in both groups. Campylobacterota were more prominent in Group 1 than in Group 2. In contrast, *Fusobacteriota*, including *Actinobacteriota*, were more abundant in Group 2 than in Group 1 (Fig. 1b). At the genus level, *Helicobacter* and *Prevotella* were more dominant in Group 1 than in Group 2. Conversely, *Bacteroides*, *Fusobacterium*, *Collinsella* and *Pseudomonas* were more abundant in Group 2 than in Group 1 (Fig. 1c). In ACE index, the Groups 1 was calculated to 389.82 for median and

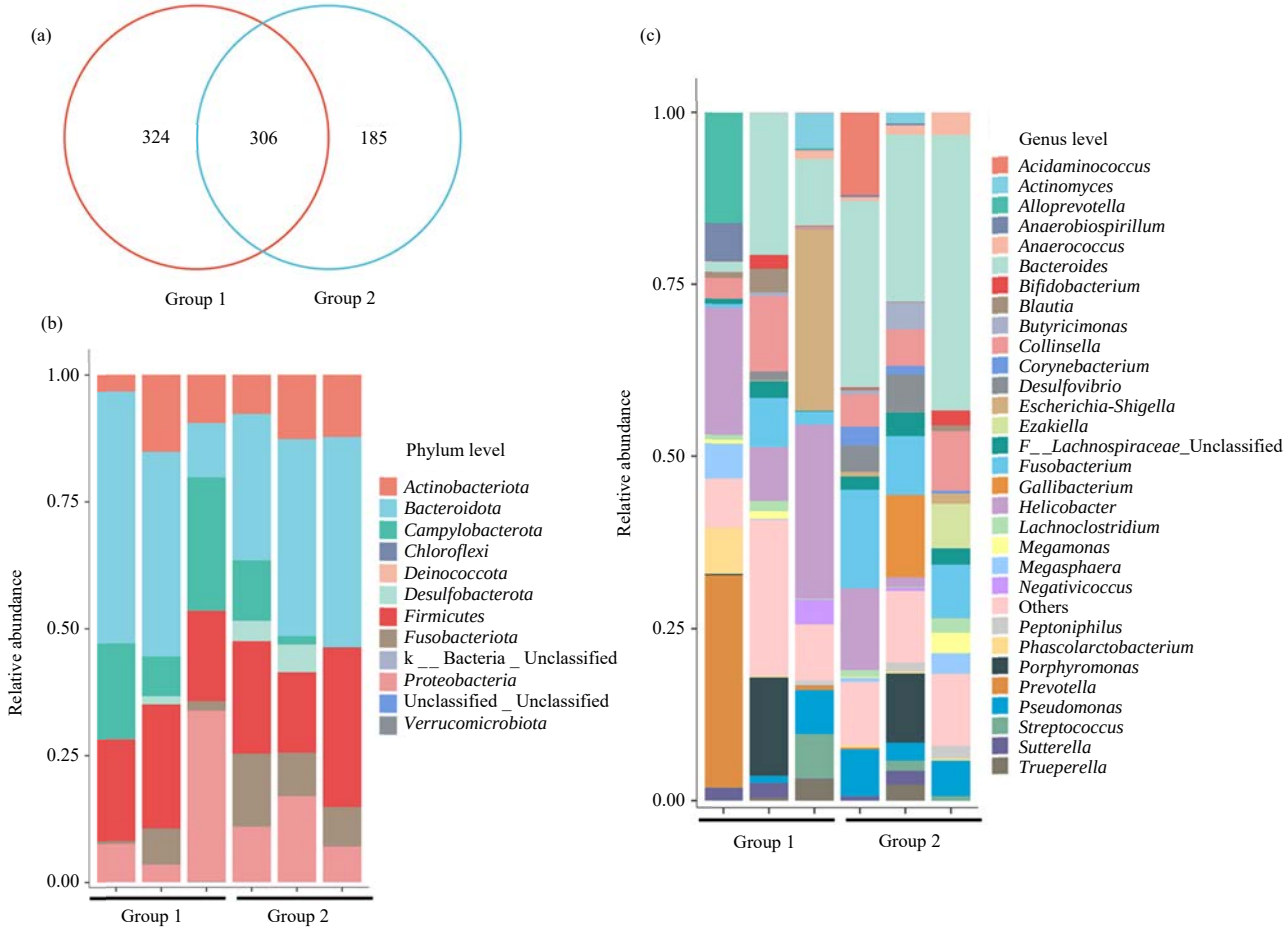

Fig. 1(a-c): Distribution of operational taxonomic units (OTUs) and bacterial composition in feline faecal samples, (a) Venn diagram showing the number of unique and shared OTUs between groups. Circles represent faecal samples from cats and numbers indicate the corresponding OTU counts, (b) Relative abundance of bacterial phyla across individual samples presented as stacked bar plots and (c) Relative abundance of bacterial genera across individual samples presented as stacked bar plots

Faecal samples containing nematode eggs were classified as Group 1 (infected cats), while samples without nematode eggs were classified as Group 2 (control/healthy cats)

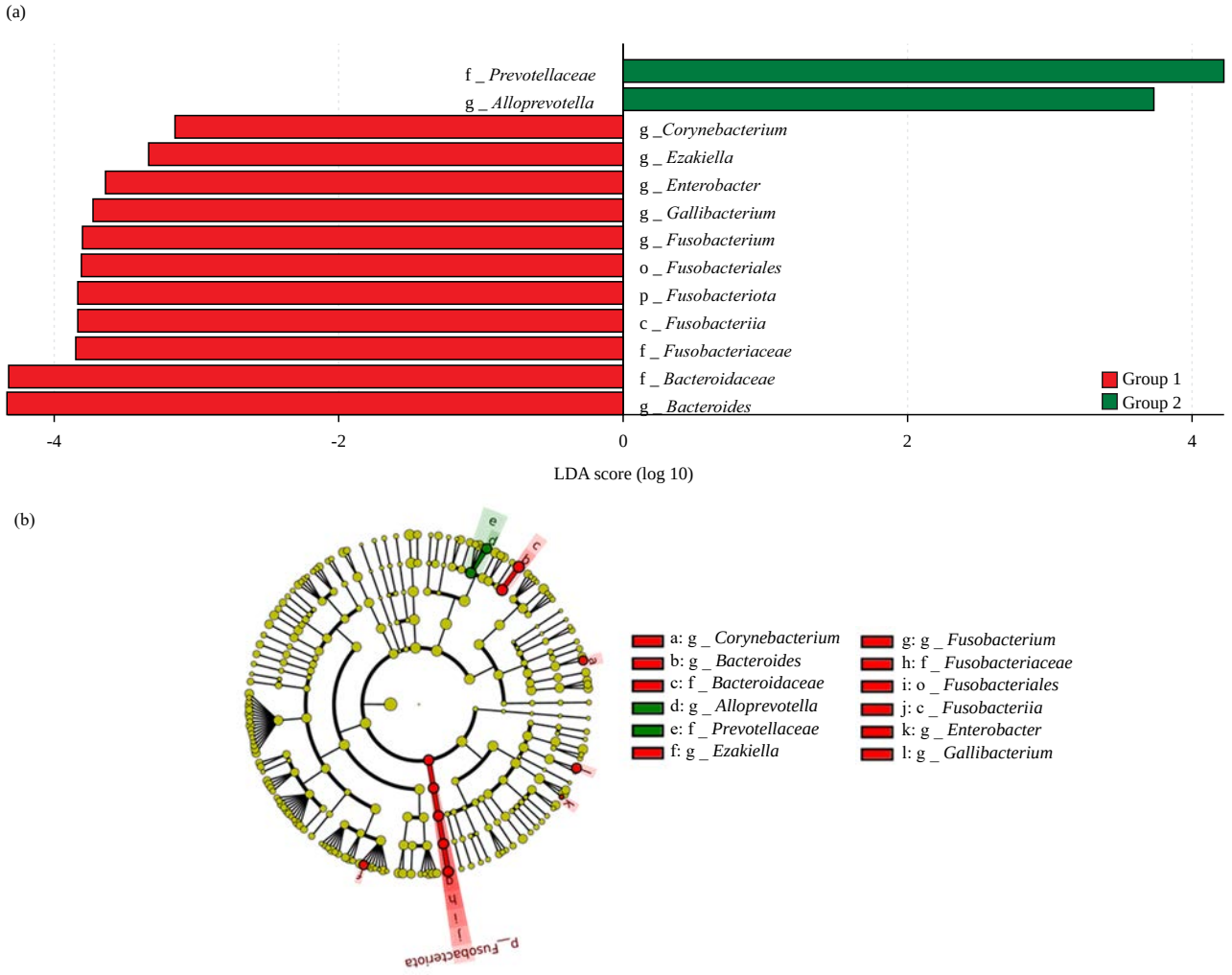

Fig. 2(a-b): Linear discriminant analysis (LDA) and phylogenetic distribution of bacterial taxa in feline faecal samples, (a) Histogram showing LDA effect size values, indicating differentially abundant bacterial taxa between groups and (b) Cladogram illustrating the phylogenetic relationships among bacterial taxa identified in the samples

Nodes with different background colours (red and green) represent taxa enriched in different groups. The corresponding taxonomic names indicated by letters are provided on the right side of the figure. Faecal samples containing nematode eggs were classified as Group 1 (infected cats), whereas samples without nematode eggs were classified as Group 2 (control/healthy cats)

112.70 for interquartile range (IQR), while Group 2 was 304.17 for median and 20.46 for IQR with 0.33 of effect size (r). In CHAO1 index, Group 1 measured as median of 377.56 with IQR of 221.18, whereas Group 2 had a median of 300.44 and an IQR of 39.97, resulting in an effect size of 0.33. In the Shannon index, Group 1 was 4.26 for median and 1.07 for IQR, but Group 2 was 4.48 median and 0.19 for IQR with a calculated effect size of 0.33. However, there was no significant difference in the alpha diversity indices between Groups 1 and 2 ($p>0.05$).

In LefSe, the taxonomic levels and biomarkers with significant differences between Groups 1 and 2 were examined. Significant variations in Group 1 included only *Alloprevotella* at the genus level. In contrast, those in Group 2 included *Bacteroides* and *Fusobacterium* at the genus level (Fig. 2a-b).

DISCUSSION

Tun *et al.*[23] reported that Bacteroidota were prevalent in faecal samples from healthy cats in South Korea. *Actinobacteria*, *Bacteroidetes*, *Firmicutes* and *Proteobacteria* were the predominant phyla in the stools of healthy felines[24]. However, Bai *et al.*[10] showed an increased abundance of Bacteroidota in acute diarrhoea in cats from China. In addition, Proteobacteria are highly prevalent biomarkers of dysbiosis

in cat[25]. In this study, Bacteroidota, followed by Firmicutes and Proteobacteria, were the dominant phyla in both groups. Group 1 was dominated by Campylobacterota, whereas Group 2 was distinguished by the predominance of *Fusobacteriota* and *Actinobacteriota*. The bacterial communities from cat stools in both groups in Thailand were partially congruent with the above reports. These results suggest that Bacteroidota, Firmicutes and Proteobacteria might be common core phyla of gut bacteria in the faecal samples of cats with or without the presence of roundworm eggs. Moreover, the results suggest that these bacteria may persist despite roundworm infection but highlight a distinct core microbiota, such as Campylobacterota, which may characterise nematode-infected felines from faecal samples.

At the genus level, *Helicobacter* was abundant in Group 1, whereas *Bacteroides*, *Fusobacterium* and *Collinsella* were abundant in Group 2. However, in LefSe, significant variation in Group 1 was *Alloprevotella*, whereas that in Group 2 was *Bacteroides* and *Fusobacterium*. *Helicobacter* is frequently associated with gastritis and diarrhoea in cats; however, it is also found in healthy cats[26]. Alessandri *et al*.[27] also reported that *Bacteroides* and *Fusobacterium* were the main bacterial populations in feline faecal samples. Furthermore, Ganz *et al*.[28] found that the high relative abundance in healthy pet cats were *Prevotella*, *Catenibacterium*, *Blautia*, *Faecalibacterium*, *Megasphaera*, *Bacteroides* and *Collinsella*. Therefore, Group 1 might favour a bacterial community with *Alloprevotella* as a biological marker, whereas high *Helicobacter* levels may confirm the presence of roundworm eggs in faecal samples. Conversely, the presence of *Bacteroides* and *Fusobacterium* in Group 2 served as biological markers, representing healthy felines.

Considering the present knowledge, it can be concluded that the bacterial community composition in cat faeces may be different or specific depending on various factors, including nematode infection in cats. Moreover, these results provide the first data on the microbiota diversity of cat faeces co-occurring with roundworm eggs. They may help support and increase our understanding of the microbiota in healthy and unhealthy feline pets.

CONCLUSION

The present study demonstrates that the bacterial community composition in feline faeces differs between cats with and without roundworm (Toxocara-like) eggs. While Bacteroidota, Firmicutes and Proteobacteria represent the dominant core microbiota in both groups, nematode-positive samples exhibited a distinct enrichment of *Campylobacterota* and *Helicobacter*, whereas *Fusobacteriota* and *Bacteroides* were more abundant in nematode-negative samples. Although overall alpha diversity did not differ significantly, differential taxa identified through LEfSe analysis suggest that helminth infection may be associated with subtle but meaningful shifts in gut microbial structure. These findings provide preliminary evidence that intestinal parasitic status is linked with alterations in the feline faecal microbiome.

SIGNIFICANCE STATEMENT

This study provides new insight into the relationship between intestinal helminth infection and gut microbiota composition in domestic cats. By comparing faecal bacterial communities in cats with and without roundworm eggs using 16S rRNA gene sequencing, the study identifies potential microbial biomarkers associated with infection status. The results contribute to a better understanding of host-parasite-microbiome interactions in felines and may support future research on microbiome-based indicators for parasitic infection and feline health monitoring.

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