Volume 10:1-14

The Undergraduate Journal of Experimental Microbiology & Immunology (+Peer Reviewed)

Dogs Are a Poor Taxonomic Model for Human Inflammatory Bowel Disease, but Are Potentially Functionally Relevant for Human Ulcerative Colitis

Brian Deng, Sharisse Chan, Renz Po, Nicholas Viegas, Max Yang

Department of Microbiology and Immunology, University of British Columbia, Vancouver, British Columbia, Canada

SUMMARY Inflammatory bowel disease (IBD) is a chronic and relapsing inflammatory condition affecting the gastrointestinal tract. Human IBD consists of two major subtypes, Crohn's Disease (CD) and Ulcerative Colitis (UC) which continue to have large global health and economic implications. Although dogs have previously been investigated as a potential research model for human IBD, their utility for specific subtypes has not been adequately considered. In this study, we explored the microbial diversity, abundance, and functional pathways of the gut microbiota in IBD dogs against UC and CD humans, to investigate if IBD dogs may serve as a research model for a particular human IBD subtype. Comparative analysis of combined 16S ribosomal RNA data from IBD dogs and humans revealed a difference in gut microbial composition between dogs and humans, further supported by a lack of common taxonomic groups in both core microbiome and indicator species analyses. Despite significant species-driven compositional differences, predictive functional pathway analysis displayed similarities in shared metabolic pathways of IBD dogs and UC humans. These findings suggest that while dogs may not serve as relatable taxonomic models for human IBD subtypes, they may hold functional relevance for human ulcerative colitis.

INTRODUCTION

Inflammatory bowel disease (IBD) is a chronic inflammatory condition affecting the gastrointestinal (GI) system and has no cure. The global prevalence of IBD continues to rise, contributing to both health-related and socio-economic burdens (1). It is estimated that by 2030, the prevalence of IBD will exceed 400,000 individuals in Canada alone, resulting in billions of dollars in healthcare costs and lost work productivity (2). IBD is currently understood as a condition amalgamated from genetic risks, environmental factors, and gut microbial dysbiosis, and can be categorized into two main subtypes: Crohn's disease (CD) and ulcerative colitis (UC) (3). CD affects the entire GI tract, while UC is typically limited to the mucosal layer of the colon (4–6).

Differences in the microbiota composition between CD and UC have been observed at the phylum level (8). These differences may stem from the unique inflammatory environments in CD and UC, which alter the nutrient landscape of the intestines and favour certain bacterial taxa (9). Functional alterations within the mucosal microbiota are also distinct between CD and UC (10). Research on the gut microbiome and host-microbe interactions to characterize their respective roles in IBD progression and pathology are ongoing, including the use of at least 66 different animal models to dissect mechanisms and develop novel therapeutics for IBD (7).

Dogs are frequently used as large animal models to investigate drug efficacy and safety, including the use of modified dog GI tracts as a model for analgesic sustained-release dosage

Published Online: September 2024

Citation: Deng, Chan, Po, Viegas, Yang. 2024. Dogs are a poor taxonomic model for human inflammatory bowel disease, but are potentially functionally relevant for human ulcerative colitis. UJEMI+ 10:1-14

Editor: Shruti Sandilya, University of British Columbia

Address correspondence to: https://jemi.microbiology.ubc.ca/

testing (11–14). Clinical, environmental, physiological, and genetic similarities make dogs suitable comparative models for human disease studies, including those related to IBD (15–17). Comparative studies have shown that the dog microbiome is more similar to the human microbiome than that of other common animal models like pigs or mice (18). The complex interplay between the gut microbiome and the immune system is also more reliably modelled by canine models than murine models (19). Both human and dog IBD conditions exhibit similar disturbances, such as decreases in microbial diversity, short-chain fatty acids (SCFAs), and secondary bile acids (BAs) (20). Additionally, the microbiomes of humans and dogs respond similarly to dietary changes, suggesting that studies using dogs may be predictive of human outcomes (21).

However, there is also evidence that challenges the reliability of dogs as models for human IBD. While cohabitation increases skin microbiota similarity between humans and dogs, it does not affect gut microbiota (22). Humans and dogs with IBD also show increased antibody binding to specific, but distinct, bacterial phyla (23). A previous study by Vázquez-Baeza *et al.* (24) found that the dysbiosis networks of IBD in dogs differ from those in humans, challenging the relevance of IBD dogs as an animal model for IBD humans. However, their analysis acknowledges human IBD more generally, without consideration of the specific CD and UC subtypes. Investigating this gap could further clarify the significance of dogs with IBD as models for specific human IBD subtypes.

Given the notable microbiota differences in individuals with CD and UC, general comparisons of human IBD to dog IBD may obscure the relevance of dog models. Therefore, we hypothesized that by comparing the microbiota of IBD dogs to the microbiota of humans with CD and UC separately, we will uncover similarities in microbiota composition and function, thus supporting the relevance of dog models for studying a specific IBD subtype.

To test this hypothesis, we examined components of the microbiota in IBD dogs and compared them to those in humans with CD and UC. We analyzed microbial diversity, taxonomic abundance, and the presence of specific taxa unique to each disease state. Additionally, we evaluated the differential abundance of microbiota species in IBD dogs relative to human IBD subtypes and compared predicted functional profiles to identify similarities in metabolic pathways.

Using the dog dataset from Vázquez-Baeza *et al*. and a human dataset obtained from Alam *et al*. (25), we found that the dog microbiota was taxonomically distinct from the human microbiota, regardless of disease state. There were minimal shared core microbiota species and no indicator taxa between IBD dogs and any human comparisons. Following this, we found that IBD dogs seem to share an overlapping functional profile with UC humans, suggesting its potential as a model for this specific subtype of IBD.

METHODS AND MATERIALS

Dataset acquisition. The dog dataset was generated by Vázquez-Baeza *et al.*, where fecal samples were analyzed from 85 healthy dogs and 65 dogs with signs of chronic gastrointestinal disease (24). IBD in dogs were diagnosed based on criteria set by the World Small Animal Veterinary Association, and 16S ribosomal RNA (rRNA) was sequenced to produce 192 samples. 15 of these samples were filtered out in downstream analyses, due to the dogs having acute haemorrhagic diarrhoea. The human dataset was obtained from Alam *et al.*, in which 9 CD patients, 11 UC patients, and 10 healthy controls had their fecal samples' 16S rRNA sequenced (25).

Data processing using the QIIME2 pipeline. The dog and human datasets were combined and demultiplexed in QIIME2 (version 2023.9) (26), followed by denoising and quality control steps which were performed in DADA2 (version 1.26.0) (27). Sequence length was truncated to 100 nucleotides per sequence, which was the maximum length of samples in the dog dataset (Figure S1). Eukaryotic samples were filtered out. The median Phred quality score at 100 nucleotides was 27. Using the Silva 138-99 database (28) trained with a truncation length of 100 and with primers 27F (AGAGTTTGATYMTGGCTCAG) and 806R (GGACTACNVGGGTWTCTAAT), the amplicon sequence variants (ASVs) were classified into individual taxonomy. QIIME2 data processing generated multiple outputs (features table,

rooted tree, taxonomy, sample metadata) that were imported into R for further downstream analysis.

Data processing in R. To facilitate data analysis using R (version 4.3.3) (29), the QIIME2 outputs were integrated into a phyloseq object using the phyloseq package (version 1.46.0) (30). Filtering steps were then conducted, removing any non-bacterial sequences and ASVs with less than five counts total, and rarefying samples using rngseed = 1 to a sampling depth of 8564 to maximize sample retention (Figure S2).

Alpha and beta diversity analyses. Alpha and beta diversity metrics were analyzed in RStudio (version 2024.04.2+764) with the following R packages: Tidyverse (version 2.0.0) (31), Vegan (version 2.6-6.1) (32), Ape (version 5.8) (33), and Phyloseq (version 1.46.0) (30). Alpha diversity metrics (Observed Features and Shannon's Diversity Index (34)) were then visualized using a boxplot with ggplot2 (version 3.5.1) (35). Statistical significance for alpha diversity metrics was computed using the 2-way analysis of variance test. The Bray-Curtis dissimilarity index (36) was also computed at a sampling depth of 8564. Statistical significance for beta diversity metrics was computed using the permutational multivariate analysis of variance (PERMANOVA) test (37). Principal coordinates analysis (PCoA) plots were generated as an output of this analysis, using the Phyloseq and ggplot2 packages.

Taxonomic barplots. Relative abundance of ASVs through Total Sum Scaling was determined in RStudio using the Tidyverse (31), Vegan (32), Ape (33), Phyloseq (30) and ggplot2 (35) packages. The average relative abundance of the top 20 families was plotted for each disease state, and as well as the average relative abundance of the top 20 genera.

Core microbiota analysis. Utilizing the Tidyverse (31), Phyloseq (30), microbiome (version 1.24.0) (38), ggvenn (39), RColorBrewer (version 1.1-3) (40), reshape2 (version 1.4.4) (41), ggplot2 (35), and knitr (version 1.48) (42) packages in RStudio, the phyloseq object was filtered by species and further filtered by disease state (i.e. healthy, IBD, UC, or CD). All subsets were transformed as compositional data. Core microbiota heatmaps (Figure S3) were generated to determine the appropriate parameters for core analysis. To remain relatively restrictive while retaining adequate core taxa, 0.00 min. relative abundance and 0.25 min. prevalence parameters were used. Core microbiota taxa were identified for each data subset. Shared and distinct core taxa between data subsets were then visualized on Venn diagrams.

Indicator species analysis. Indicator species analysis identified the taxonomic groups significantly associated with each disease state in humans and dogs. The Phyloseq object was transformed into compositional relative abundance, and grouped to either the genus or family level. Utilizing the Indicspecies (version 1.7.14) (43), Tidyverse (31) and Phyloseq (30) packages in RStudio, both disease states of humans and dogs and species were used as predictors within the multipatt function for analysis. Species were filtered for significance with a p-value lesser than 0.05.

Differential abundance analysis. Taxa that differed in abundance between various disease states were identified using the DESeq2 (version 1.42.1) (44), Tidyverse (31) and Phyloseq (30) packages in RStudio. The CD and UC humans were defined as the reference groups for comparisons with IBD dogs, and significantly differentially abundant taxa were filtered according to padj<0.01 and |log2FoldChange|>2. The top ten hits for positive and negative differentially abundant genera were represented in bar plots using ggplot2 (35).

Predictive functional analysis. After filtering out features with five or lower counts, PICRUSt2 (45) was installed in the local terminal and ran to conduct hidden-state prediction of genomes, metagenome prediction, and pathway-level predictions. The metadata and abundance files from this analysis were then imported to R for further processing. Using packages readr (46), ggpicrust2 (45), Tibble (47), Tidyverse (31), ggprism (48), patchwork (49), ggh4x (50), and dplyr (51), a custom function was created to summarize results from the differential abundance analysis of the functional pathways using the DESeq2 method (44).

Principal Component Analysis (PCA) plots were then created to visualize the relationship between our different samples based on their functional portfolios using ggpicrust2 (45).

RESULTS

The human and dog microbiota are compositionally diverse. To explore the differences in the microbial diversity between humans and dogs with different disease states, we characterized the alpha diversity of their microbiota samples through observed features and Shannon's Diversity Index. The human gut microbiota samples were significantly richer (Figure 1A) and more abundant and even (Figure 1B) in comparison to dogs. These results are consistent when comparing healthy dogs to healthy humans and dogs with IBD to humans with Crohn's Disease and Ulcerative Colitis. We also performed Beta diversity analysis, looking into the Bray-Curtis dissimilarity index, to illustrate how compositionally different the human and dog gut microbiotas are. Here, we observed a similar trend with our alpha diversity analysis, where humans and dogs are significantly different, regardless of disease state (Figure 1C). Statistical testing using PERMANOVA indicated both species and disease state drive significant differences associated with beta diversity when characterized using the Bray-Curtis dissimilarity index.

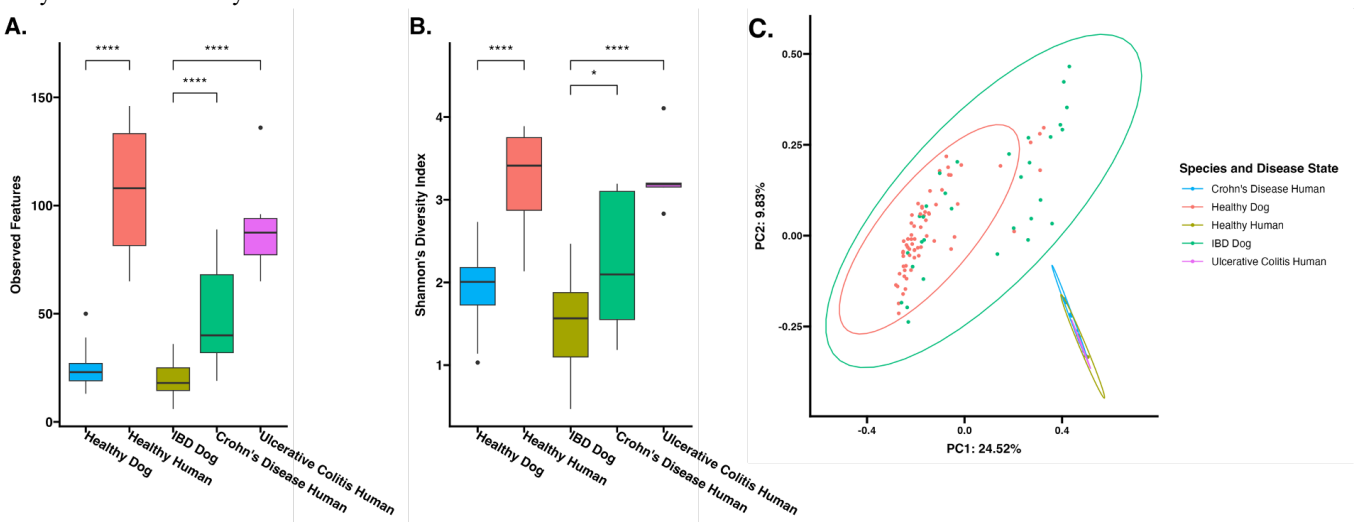

FIG. 1 The human gut microbiota shows significantly greater richness and higher abundance, and is compositionally different from the dog microbiota. (A) Observed features were used to measure richness while (B) Shannon's Diversity Index was used to measure abundance and evenness in the human and dog microbiotas. Box plots were used for visualization and error bars represent mean ± SEM. Statistical analysis was performed via 2-way analysis of variance test. P-values are denoted on the respective plots (* $p < 0.05$, **** $p < 0.0001$). (C) Principal Coordinate Analysis (PCoA) plot of beta diversity looking into the Bray-Curtis dissimilarity index for dogs and humans with different disease states ($p = 0.0001$, PERMANOVA). Ellipses represent a 95% confidence interval.

The human and dog microbiota are taxonomically distinct. We subsequently explored taxonomic differences to corroborate the observed compositional distinctiveness. Taxonomic barplots were generated to illustrate the relative abundance of microbes at the family and genus levels in humans and dogs, with groupings being made based on disease state. Across humans, Lachnospiraceae was the most well represented family, while dogs showed a lower relative abundance of Lachnospiraceae and had highest relative abundance of Erysipelotrichaceae (Figure 2A). Compared to healthy humans, CD humans exhibited higher relative abundances of Veillonellaceae and Enterobacteriaceae, as well as the presence of Prevotellaceae in both CD and UC. IBD dogs displayed reduced relative abundance of Leuconostocaceae and Prevotellaceae, and increased Peptostreptococcaceae, Glycomycetaceae, and an uncultured family from the Clostridiales order, compared to healthy dogs. At the genus level, the difference between dog and human microbiota composition is more pronounced (Figure 2B). The dog microbiota is dominated by *Catenisphaera*, while the human microbiota contains a wider variety of genera. Of note, there is higher relative abundance of *Prevotella* and *Anaerostipes* in CD and UC humans compared to healthy

humans, while IBD dogs showed a decreased relative abundance of *Prevotella* and an increase in an uncultured genus from Clostridiales compared to healthy dogs.

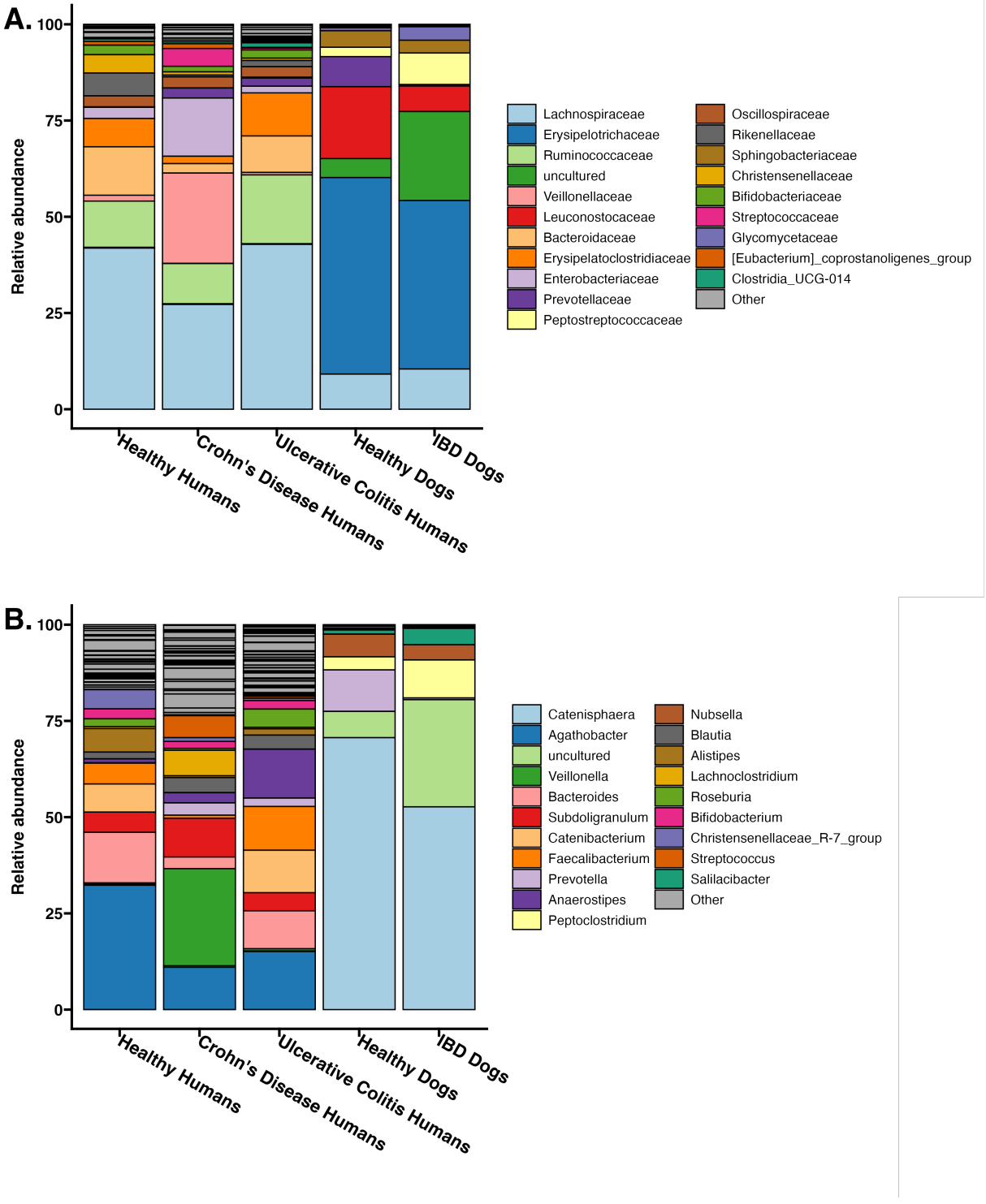

FIG. 2 There is variation in relative abundance levels of taxa between dogs and humans, and their conditions. Taxonomic bar blots were generated, showing Total Sum Scaling relative abundance of taxa at the **(A)** family level and **(B)** the genus level, across different species and disease statuses. Only the top 20 most relatively abundant taxa are coloured in each plot, with other taxa pooled into the "Other" category.

Core microbiota and indicator species analyses show key microbial players are distinct in relation to dog and human IBD conditions. After analyzing the core microbiota, we found that IBD dogs, UC humans, and CD humans consisted of 5, 51, and 33 ASVs, respectively, prior to Venn diagram comparison. Following comparison, we found only one common core taxa between all three conditions, belonging to the *Lachnospiraceae* family (Figure 3). Our indicator species analysis demonstrated that this taxon is not an indicator species in dogs, regardless of health, while being an indicator species in all human disease states. Additionally, we uncovered similar patterns of differing microbial composition between dogs and humans. A larger comparison between indicator taxa resolved to the genus level between human and dog species revealed 9 species specific to dogs and 130 specific to humans, with no shared genera. When looking specifically at each disease state resolved to a genus level, including healthy dogs, IBD dogs, UC humans, CD humans, and healthy humans, there were no indicator genera for either IBD or healthy dogs, despite there being 11 indicator genera for UC, 9 for CD and 24 for healthy humans (Table S1). Our results demonstrate a contrast in core microbiota composition and indicator species depending on whether the sample came from a dog or human, regardless of the disease state.

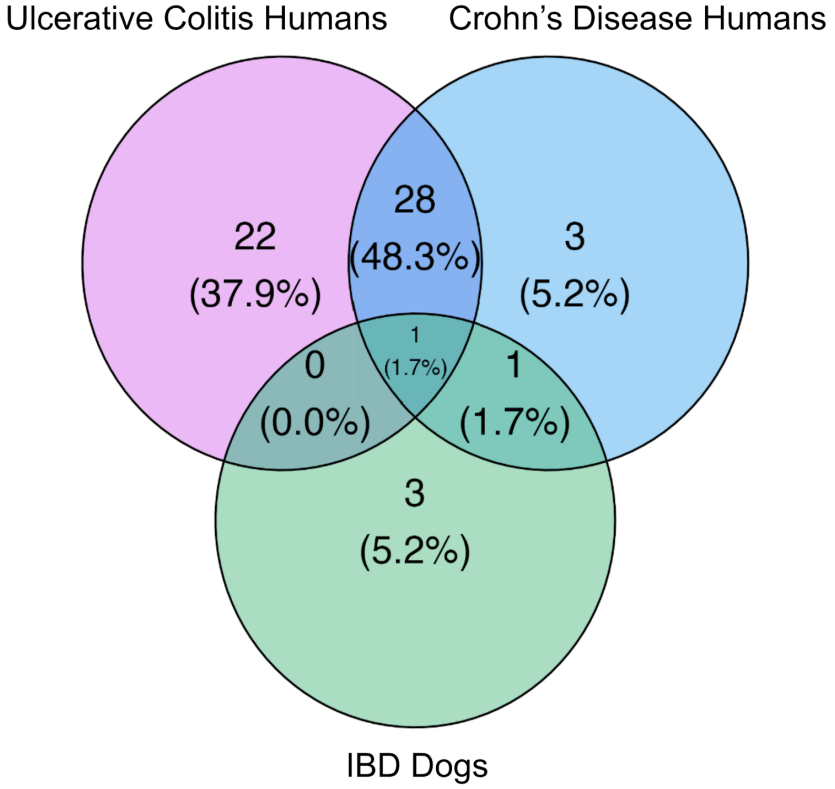

FIG. 3 Relative proportions of compared core microbiomes indicate little to no common core taxa between dog IBD and either or both human disease states. Core microbiomes were identified using a minimum abundance of 0% and minimum prevalence of 25%. The core taxa were resolved at the genus level.

The IBD dog microbiota is differentially abundant from both the human CD and UC microbiota. To identify significant alterations in representative taxa between IBD dogs and CD or UC humans, we conducted differential abundance analysis using DESeq2. We observed a significant reduction ($p_{adj} \leq 0.01$) in differentially abundant taxa in IBD dogs, when compared to the human disease states. Taxa in dogs were generally less representative when compared to humans, with 109 ASVs less representative in IBD dogs relative to CD humans (Figure 4A), and 147 ASVs less representative in relation to UC humans (Figure 4B). 41 and 44 ASVs were more representative in IBD dogs compared to CD and UC humans, respectively. Of genera with the highest magnitude of log2FoldChange in IBD dogs, we found that the genera of *Catenisphaera* and *Ruminococcus gnavus group* were more representative in their microbiota when compared to both CD and UC humans (Figure 4C-D). Lastly, the magnitude of log2 fold change when comparing any dog microbiota to a human is up to four times greater than that of comparisons made between states of the human microbiota (Figure S4A-B).

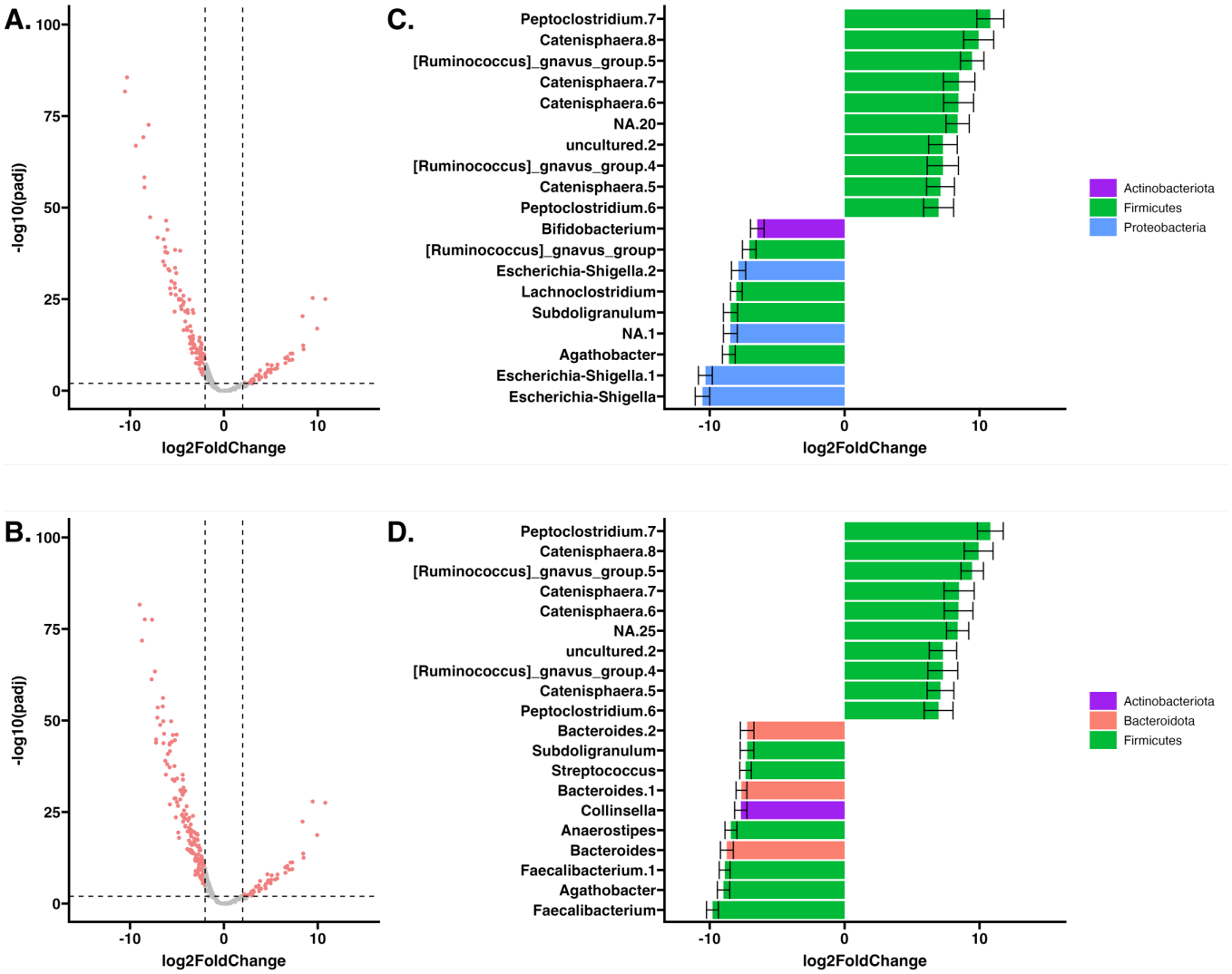

FIG. 4 Significant differential abundance of the IBD dog microbiome in relation to CD humans and UC humans. Volcano plots display significantly differentially abundant taxa in terms of the magnitude of fold changes (|log2FoldChange| > 2) and high statistical significance ($p_{adj} < 0.01$) coloured in pink, evaluated in IBD dogs in relation to **(A)** CD humans and **(B)** UC humans. Taxa bar plots illustrating the top 10 hits of differentially abundant genera in IBD dogs relative to **(C)** CD humans and **(D)** UC humans are also shown, with bars coloured by phylum.

Predictive functional analysis demonstrates that dogs with IBD appear to be functionally similar to humans with UC. Since different microbial species may have similar functions, we performed a predictive functional analysis using PICRUSt2 to evaluate whether metabolic pathways may be similar between different groups. This analysis showed that healthy humans and healthy dogs are diverse in functional potential, with minimal overlap observed in the PCA plot (Figure 5A). A similar trend was observed in IBD dogs and CD humans (Figure 5B). When comparing IBD dogs to UC humans, similar functional pathways in branched-chain amino acid (BCAA) synthesis pathways appeared to be shared between the two groups (Figure S5), with the human cluster completely within the dog cluster in the PCA plot (Figure 5C).

DISCUSSION

Using alpha diversity analyses, we found that the human gut microbiota, regardless of disease state, demonstrated significantly higher richness in observed features when compared to their dog counterparts (Figure 1A). A similar trend is observed when considering community abundance and evenness through Shannon's Diversity Index (Figure 1B).

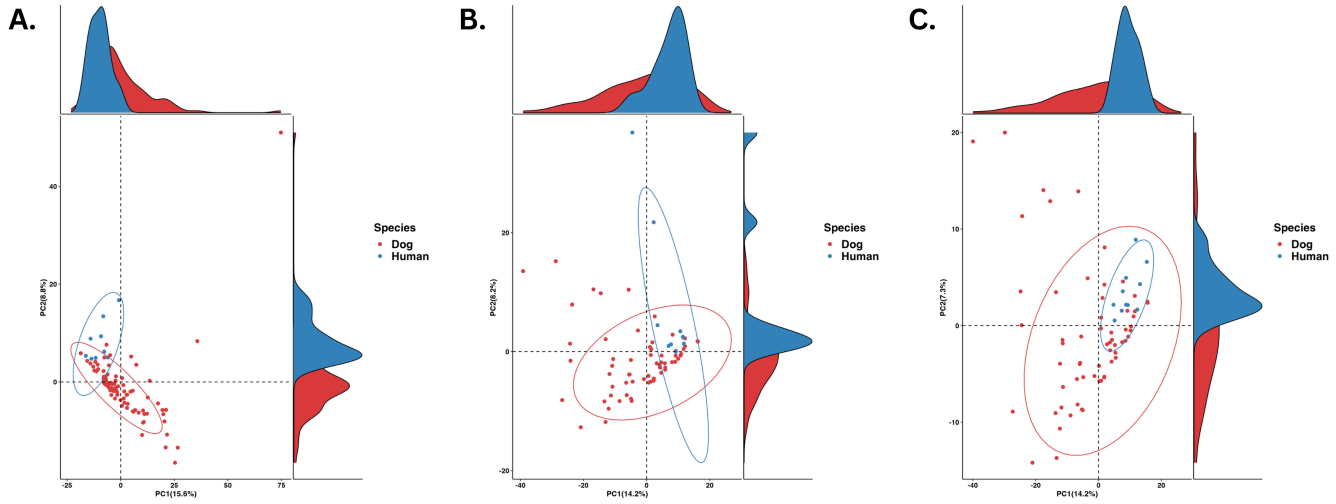

FIG. 5 Predictive functional analysis demonstrates that dogs with IBD appear to be functionally similar to humans with UC. Principal Component Analysis (PCA) plots show similarities in -predicted functional potential of (A) healthy humans and healthy dogs, (B) dogs with IBD and humans with Crohn's disease, and (C) dogs with IBD and humans with Ulcerative Colitis. Ellipses represent a 95% confidence interval.

Overall, this implies that humans have a greater variety of microbial species found in the gut compared to dogs, with studies comparing the phylogenetic diversity of humans and dogs showing consistent results (24). Although IBD dogs have significantly lower Shannon's Diversity Index compared to healthy dogs (p = 0.0002), indicating reduced microbial abundance and evenness, the samples did not significantly differ in richness (p = 0.5). The reduced Shannon's Diversity Index for dogs affected by IBD were reproduced by Calalang *et al.* (52) and is consistent with the overall reduced microbial diversity found in human IBD patients from other studies (53). As a decreasing trend in abundance is not replicated when looking at richness, our study highlights the importance of utilizing different metrics of alpha diversity to better understand how the gut microbiota is affected by IBD. Moreover, humans having higher alpha diversity metrics suggests that the human gut microbiota is more complex when considering the number of different species and their associated relative abundance. Beta diversity analysis, utilizing the Bray-Curtis dissimilarity index, highlighted that the gut microbial communities cluster based on species (Figure 1C), as opposed to disease. Taken together, this suggests that dogs are not a good taxonomic model for IBD in humans, as the microbial composition and diversity in dogs may not fully capture the complexity seen in humans.

Subsequent analysis of the taxonomic composition of the microbiotas of dogs, humans, and their respective disease statuses points towards further differences. Across both family and genus, there are clear species-driven differences in taxa (Figure 2). Between all humans and healthy dogs, the only noticeable shared genus belongs to *Prevotella*, while its relative abundance is reduced in IBD dogs altogether. *Prevotella* bacteria are known producers of beneficial and anti-inflammatory SCFAs, and its reduction in IBD dogs is consistent with other studies (54). The dog microbiota also contains a large proportion of *Catenisphaera* in both healthy and IBD dogs, a novel, minimally characterized genus first isolated in a Japanese anaerobic digester (55). The IBD dog microbiota contains a larger proportion of an uncultured genus from the Clostridiales order relative to healthy dogs. The Clostridiales order contains a wide variety of bacteria, from SCFA-producing microbes like *Faecalibacterium* to those associated with IBD like *Ruminococcus gnavus* (56–59). Further characterization of the dog microbiota is needed to identify the significance of representative genera like *Catenisphera* and other uncultured bacteria. Overall, the stark taxonomic contrast between humans and dogs as a specifies suggests that the human IBD microbiota may not be fully appreciated using a dog model of disease.

The dissimilarities in gut microbiota composition are further amplified by our analyses on the core microbiota and indicator species, which highlight that key microbiota taxa in IBD dogs and either IBD human subtype are not similar. Our core microbiota (Figure 3) and indicator species (Table S1) analyses show that the core and indicative taxa for each specific disease state are not parallel, whereas the gut microbiota are more influenced by species than by the health condition. There is a single core taxa present in all dog and human IBD conditions, belonging to the *Lachnospiraceae* family, which has previously been linked to IBD (60). However, our indicator species analysis does not validate this core taxa as an indicator in dogs, whether or not diagnosed with IBD. Thus, our results suggest that there are little to no shared core genera between IBD dogs and any of the IBD human disease states, nor are there any indicator species for either IBD dogs or healthy dogs. While there is a lack of shared taxonomic groups between the disease states in humans and dogs, the majority of indicator genera for human IBD have previously been studied to be associated with IBD such as *Desulfovibrio* (61) and *Sutterella* (62). The absence of these human IBD genera and any other indicator taxa in IBD and healthy dogs highlights the influence of species on driving the presence or absence of key microbial players, rather than the IBD conditions.

The difference in microbial compositions between dogs and humans is further supported by our differential abundance analysis, which revealed that the dog microbiota is significantly less abundant than the human microbiota. In reference to both CD and UC humans, more than double the number of more representative taxa in IBD dogs were significantly less representative (Figure 4A-B). In addition, there were minimal differences in the representative genera of the dog microbiotas between the different comparisons in human disease state reference groups (Figure 4C-D). This suggests a relatively simpler microbiota with less overall microbial abundance in dogs and is supported by our findings in the relative abundance of genera in dog species (Figure 2B). Diet shapes the gastrointestinal microbiota, and most humans and dogs consume different diets, in that common dog foods are kibbles in contrast to the more complex and processed foods that humans consume (63). Consumption of more complex foods such as non-digestible carbohydrates can lead to increases in gut microbe diversity (64), and the much more diverse human gut microbiota only overlaps by 3.2% with the dog microbiota in terms of gene pool (21). Although similar studies employing these analytical tools and sample species are limited, our findings on distinct microbial dysbiosis between dogs and humans are consistent with Vázquez-Baeza *et al*. (24). This is evident in our differential abundance analyses that reveal comparable discrepancy between healthy and IBD dogs in reference to healthy humans (Figure S4C-D), and to CD (Fig 5A) and UC (Fic 5B) humans. Our findings suggest that the species influence on gut microbiota composition is a more significant factor than the influence of whether the subject has IBD, further discouraging the use of IBD dogs as a taxonomic model for human IBD.

Given the species-driven differences in microbial diversity, the taxonomic composition between dogs and humans may be less relevant for a disease model, as common metabolic pathways that can be fulfilled by multiple species are involved in disease susceptibility. Predictive functional pathway analysis using PICRUSt2 allowed us to characterize the differentially expressed enzymes across various disease states within dogs and humans. The resulting PCA plots of this analysis illustrated differing metabolic pathways between healthy dogs and healthy humans (Figure 5A), as well as IBD dogs and CD humans (Figure 5B). However, IBD dogs and UC humans seem to share some similar predicted functional potential (Figure 5C). Significance in these shared pathways implies that these metabolic functions are conserved across humans and dogs and can indicate that these are essential microbial processes for the functioning of microbial communities present in the gut. While previous studies highlight that the most abundant pathways shared between humans and dogs are housekeeping genes that are not associated with disease (24), our functional analysis is inconsistent with this notion. Specifically, one pathway of interest that is shared between IBD dogs and UC humans is the synthesis of BCAAs (Figure S5), which has been implicated in predicting the response of humans to anti-integrin therapy for IBD (65). Studies show that BCAAs may reduce colonic inflammation by upregulating human beta-defensin 1 in colonic cells, of which expression is altered in IBD pathogenesis (66). Clinical studies have also illustrated that BCAAs play a fundamental role in maintaining intestinal health, supporting the significance of BCAAs as emerging biomarkers in UC (67). Accordingly, this shared

pathway between IBD dogs and UC humans render dogs functionally relevant for studying the UC subtype. However, more research is needed to assess the utility of dogs as functional models of human UC.

Despite functional similarities in IBD dogs and UC humans, our analysis demonstrated that other metabolic pathways also serve as key factors in human incidences of UC but are not shared with dogs (Figure S5). These include L-arginine biosynthesis and pathways involved in lipid metabolism. Serum L-arginine levels were shown to correlate with disease severity in human studies of UC (68). However, our research suggests that neither L-arginine biosynthesis pathways are significantly altered in the dog microbiota (Figure S5). Moreover, metabolic profiling of human samples shows that lipid metabolism is also significantly altered in UC (69). Growing evidence suggests that lipid metabolism plays an important role in the pathogenesis of IBD (70) and that there is potential for lipid and metabolic profiling to become diagnostic tools of IBD, differentiating between CD and UC (71). Because these different lipid metabolism pathways have not been included in the shared functional pathways between IBD dogs and humans with UC, the potential of dogs as functional models for UC is shown to be rather complex. This suggests that while BCAA metabolism is a shared pathway that has been implicated in disease, further studies are required to explore whether dogs affected with IBD are suitable functional models for human UC.

Limitations The main limitation of this study was the use of two different datasets to conduct our analyses. The data was obtained from two different studies, which themselves were based upon inconsistent methodologies, such as distinct sample collection procedures, collection time, and sample size. In regards to sample collection, leftover fecal samples were collected from dogs participating in other clinical studies (24), while human fecal samples were first frozen before use (25). Collection time varied between the two groups, with the first-morning samples being collected from humans and the dog samples having non-specified collection time points. These differences can lead to protocol-dependent biases, such as certain taxa being preferentially amplified by different PCR primers (72)

The sample sizes between the two datasets used for this study also differed, with the dog dataset providing a much larger sample size (160 total samples) in comparison to humans (16 total samples) after rarefaction. The Phred quality score in the dog dataset is lower in comparison to that of the human dataset, and the sequence length of the combined dataset was truncated to 100 nucleotides as per the maximum base length of the dog dataset (Figure S1). A lower Phred score corresponds to a higher probability of an incorrect base call, so the quality and length of the combined dataset are limited by the dog dataset. Furthermore, only the disease status and species were combined in the metadata file due to non-uniform variables across the datasets, and other confounding variables were not considered in our analyses. Previous research illustrates that factors such as age and antibiotic treatment impact the dog microbiota (73), and not accounting for these factors in our research impacts our ability to make conclusions about the taxonomic composition and predicted function of the dog microbiota.

We acknowledge that such limitations are present in our study, but we have laid down the groundwork and conducted novel analyses to open up this field to more research.

Conclusions Our study aimed to evaluate the microbial diversity, composition, and function of the IBD dog microbiota as a potential model for the human IBD subtypes of CD and UC. We compared alpha and beta diversity, relative and differential abundance of taxa, the core microbiota and indicator species, as well as predicted functional pathways between the disease states of dogs and humans. Our results confirm that the dog microbiota is less diverse and taxonomically distinct from the human microbiota, regardless of disease state. Despite taxonomic differences, there is shared predicted functional potential between IBD dogs and UC humans, notably in BCAA synthesis pathways. IBD dogs may not serve as a reliable taxonomic model for humans with IBD, but there is potential for IBD dogs as a functional model for UC humans, such as for testing therapeutics like anti-integrin therapy for IBD, which involves BCAA pathways.

Future Directions This study provides a proof of concept for further investigation into how dogs affected with IBD are a poor taxonomic model for human IBD but may be a functionally relevant model for humans with ulcerative colitis. To address one of this study's major limitations, a similar study can be conducted using the same sample collection and DNA extraction protocol for both humans and dogs with IBD. By streamlining methodologies, controls can be made for confounding variables such as collection methods, and sample size, and DNA yield. Conducting a similar study with a higher quantity of more robust samples would also elucidate more substantial results, allowing for verification of the trends we observed in our taxonomic and functional analyses.

Additional investigation into the differences in individual traits, such as age and breed, in both humans and dogs would allow for a stronger characterization of taxonomic and functional differences between various groups. This is particularly important as age is a crucial factor driving the gut microbial community of dogs, with the abundance of some species demonstrating a positive correlation with age (74). The microbial composition of dogs is also shown to be significantly affected by breed. This is demonstrated in studies showing that Fusobacteria are the dominant phylum in Maltese while Firmicutes and Actinobacteria are more abundant in Poodles provided with similar housing conditions and diet (75).

There is a large proportion of the dog microbiota that remains uncultured and unable to be identified (Figure 2B). Further characterization of these uncultured species will provide insights into its taxonomic composition, and more importantly, its functionality relative to human UC. Similarly, further studies must be done to elucidate the significance of *Catenisphaera* in the dog microbiota, and its relevance in IBD. These findings will further our understanding of the dog microbiota, and its relevance as a potential functional model for human UC.

Lastly, we would conduct further metabolome analyses to examine metabolic alterations caused by IBD in dogs and humans. This will allow for a more robust characterization of the functional similarities and differences in dogs with IBD and humans with UC, providing further insight to the potential of using dogs as models. In vivo studies with the appropriate controls must also be pursued to support findings from metabolic analyses, as previous studies have demonstrated that taxa-function variation arises due to differences in the external environment (74). Examples of further studies include using dog models to investigate responses to different IBD therapeutics and comparing this to studies conducted in humans. This could provide more insight towards the translatability of dog models, such as whether the observed metabolic similarities would allow us to predict human drug responses from dog models.

ACKNOWLEDGEMENTS

The authors acknowledge that the land we performed this research on is the traditional, ancestral, and unceded territory of the xwməθkwəy̓əm (Musqueam) Nation. We encourage others to learn more about the native lands in which they live and work at https://native-land.ca/. The authors would like to thank Dr. Evelyn Sun, Christopher Lee, Avril Metcalfe-Roach, and the rest of the MICB 475 teaching team for their invaluable mentorship and guidance throughout this project, and the UBC Department of Microbiology and Immunology for its funding and resources. The authors give gratitude to Vázquez-Baeza *et al*. and Alam *et al.* for providing the datasets that were used in our study, as well as the reviewers for their constructive feedback.

CONTRIBUTIONS

All authors were involved in conceptualization, formal data analysis in RStudio, as well as writing and editing of the manuscript. B.D. and R.P. performed formal data analysis in QIIME2.

REFERENCES

1. **Caviglia GP, Garrone A, Bertolino C, Vanni R, Bretto E, Poshnjari A, Tribocco E, Frara S, Armandi A, Astegiano M, Saracco GM, Bertolusso L, Ribaldone DG.** 2023. Epidemiology of

Inflammatory Bowel Diseases: A Population Study in a Healthcare District of North-West Italy. *J Clin Med* **12**:641.

2. **Kaplan GG, Bernstein CN, Coward S, Bitton A, Murthy SK, Nguyen GC, Lee K, Cooke-Lauder J, Benchimol EI.** 2019. The Impact of Inflammatory Bowel Disease in Canada 2018: Epidemiology. *J Can Assoc Gastroenterol* **2**:S6–S16.
3. **Actis GC, Pellicano R, Fagoonee S, Ribaldone DG.** 2019. History of Inflammatory Bowel Diseases. *J Clin Med* **8**:1970.
4. **Shan Y, Lee M, Chang EB.** 2022. The Gut Microbiome and Inflammatory Bowel Diseases. *Annu Rev Med* **73**:455–468.
5. **Le Berre C, Ananthakrishnan AN, Danese S, Singh S, Peyrin-Biroulet L.** 2020. Ulcerative Colitis and Crohn's Disease Have Similar Burden and Goals for Treatment. *Clin Gastroenterol Hepatol* **18**:14–23.
6. **Cerquetella M, Spaterna A, Laus F, Tesei B, Rossi G, Antonelli E, Villanacci V, Bassotti G.** 2010. Inflammatory bowel disease in the dog: Differences and similarities with humans. *World J Gastroenterol WJG* **16**:1050–1056.
7. **Mizoguchi A.** 2012. Animal Models of Inflammatory Bowel Disease, p. 263–320. *In* Conn, PM (ed.), Progress in Molecular Biology and Translational Science. Academic Press.
8. **Forbes JD, Van Domselaar G, Bernstein CN.** 2016. Microbiome Survey of the Inflamed and Noninflamed Gut at Different Compartments Within the Gastrointestinal Tract of Inflammatory Bowel Disease Patients. *Inflamm Bowel Dis* **22**:817–825.
9. **Stecher B.** 2015. The Roles of Inflammation, Nutrient Availability and the Commensal Microbiota in Enteric Pathogen Infection. *Microbiol Spectr* **3**:10.1128/microbiolspec.mbp-0008–2014.
10. **Davenport M, Poles J, Leung JM, Wolff MJ, Abidi WM, Ullman T, Mayer L, Cho I, Loke P.** 2014. Metabolic Alterations to the Mucosal Microbiota in Inflammatory Bowel Disease. *Inflamm Bowel Dis* **20**:723–731.
11. **Ambrosini YM, Borcherding D, Kanthasamy A, Kim HJ, Willette AA, Jergens A, Allenspach K, Mochel JP.** 2019. The Gut-Brain Axis in Neurodegenerative Diseases and Relevance of the Canine Model: A Review. *Front Aging Neurosci* **11**:130.
12. **Potschka H, Fischer A, von Rüden E-L, Hülsmeyer V, Baumgärtner W.** 2013. Canine epilepsy as a translational model? *Epilepsia* **54**:571–579.
13. **Loen V, Vos MA, van der Heyden MAG.** 2022. The canine chronic atrioventricular block model in cardiovascular preclinical drug research. *Br J Pharmacol* **179**:859–881.
14. **Yamada K, Furuya A, Akimoto M, Maki T, Suwa T, Ogata H.** 1995. Evaluation of gastrointestinal transit controlled-beagle dog as a suitable animal model for bioavailability testing of sustained-release acetaminophen dosage form. *Int J Pharm* **119**:1–10.
15. **Hytönen MK, Lohi H.** 2016. Canine models of human rare disorders. *Rare Dis* **4**:e1241362.
16. **Kirkness EF, Bafna V, Halpern AL, Levy S, Remington K, Rusch DB, Delcher AL, Pop M, Wang W, Fraser CM, Venter JC.** 2003. The dog genome: survey sequencing and comparative analysis. *Science* **301**:1898–1903.
17. **Hashimoto-Hill S, Alenghat T.** 2021. Inflammation-Associated Microbiota Composition Across Domestic Animals. *Front Genet* **12**.
18. **Pallotti S, Piras IS, Marchegiani A, Cerquetella M, Napolioni V.** 2022. Dog–human translational genomics: state of the art and genomic resources. *J Appl Genet* **63**:703–716.
19. **Kleber KT, Iranpur KR, Perry LM, Cruz SM, Razmara AM, Culp WTN, Kent MS, Eisen JA, Rebhun RB, Canter RJ.** 2022. Using the canine microbiome to bridge translation of cancer immunotherapy from pre-clinical murine models to human clinical trials. *Front Immunol* **13**:983344.
20. **Hernandez J, Rhimi S, Kriaa A, Mariaule V, Boudaya H, Drut A, Jablaoui A, Mkaouar H, Saidi A, Biourge V, Borgi MA, Rhimi M, Maguin E.** 2022. Domestic Environment and Gut Microbiota: Lessons from Pet Dogs. *Microorganisms* **10**.
21. **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.
22. **Song SJ, Lauber C, Costello EK, Lozupone CA, Humphrey G, Berg-Lyons D, Caporaso JG, Knights D, Clemente JC, Nakielny S, Gordon JI, Fierer N, Knight R.** 2013. Cohabiting family members share microbiota with one another and with their dogs. *eLife* **2**:e00458.
23. **Soontararak S, Chow L, Johnson V, Coy J, Webb C, Wennogle S, Dow S.** 2019. Humoral immune responses against gut bacteria in dogs with inflammatory bowel disease. *PLoS ONE* **14**:e0220522.
24. **Vázquez-Baeza Y, Hyde ER, Suchodolski JS, Knight R.** 2016. Dog and human inflammatory bowel disease rely on overlapping yet distinct dysbiosis networks. 12. *Nat Microbiol* **1**:1–5.
25. **Alam MT, Amos GCA, Murphy ARJ, Murch S, Wellington EMH, Arasaradnam RP.** 2020. Microbial imbalance in inflammatory bowel disease patients at different taxonomic levels. *Gut Pathog* **12**:1.
26. **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, Kosciolek T, Kreps J, Langille MGI,**

Lee J, Ley R, Liu Y-X, Loftfield E, Lozupone C, Maher M, Marotz C, Martin BD, McDonald D, McIver LJ, Melnik AV, Metcalf JL, Morgan SC, Morton JT, Naimey AT, Navas-Molina JA, Nothias LF, Orchanian SB, Pearson T, Peoples SL, Petras D, Preuss ML, Pruesse E, Rasmussen LB, Rivers A, Robeson MS, Rosenthal P, Segata N, Shaffer M, Shiffer A, Sinha R, Song SJ, Spear JR, Swafford AD, Thompson LR, Torres PJ, Trinh P, Tripathi A, Turnbaugh PJ, Ul-Hasan S, van der Hooft JJJ, Vargas F, Vázquez-Baeza Y, Vogtmann E, von Hippel M, Walters W, Wan Y, Wang M, Warren J, Weber KC, Williamson CHD, Willis AD, Xu ZZ, Zaneveld JR, Zhang Y, Zhu Q, Knight R, Caporaso JG. 2019. Reproducible, interactive, scalable and extensible microbiome data science using QIIME 2. *Nat Biotechnol* **37**:852–857.

27. **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.
28. **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.
29. **R Core Team.** 2023. R: A Language and Environment for Statistical Computing. R Foundation for Statistical Computing, Vienna, Austria.
30. **McMurdie PJ, Holmes S.** 2013. phyloseq: An R Package for Reproducible Interactive Analysis and Graphics of Microbiome Census Data. *PLOS ONE* **8**:e61217.
31. **Wickham H, Averick M, Bryan J, Chang W, McGowan LD, François R, Grolemund G, Hayes A, Henry L, Hester J, Kuhn M, Pedersen TL, Miller E, Bache SM, Müller K, Ooms J, Robinson D, Seidel DP, Spinu V, Takahashi K, Vaughan D, Wilke C, Woo K, Yutani H.** 2019. Welcome to the Tidyverse. *J Open Source Softw* **4**:1686.
32. **Oksanen J, Blanchet FG, Kindt R, Legendre P, Minchin P, O'Hara B, Simpson G, Solymos P, Stevens H, Wagner H.** 2015. Vegan: Community Ecology Package. *R Package Version 22-1* **2**:1–2.
33. **Paradis E, Claude J, Strimmer K.** 2004. APE: Analyses of Phylogenetics and Evolution in R language. *Bioinformatics* **20**:289–290.
34. **Ortiz-Burgos S.** 2016. Shannon-Weaver Diversity Index, p. 572–573. *In* Kennish, MJ (ed.), Encyclopedia of Estuaries. Springer Netherlands, Dordrecht.
35. **Wickham H.** 2009. ggplot2: Elegant Graphics for Data Analysis. Springer, New York, NY. https://link.springer.com/10.1007/978-0-387-98141-3. Retrieved 6 December 2023.
36. **Sørensen 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 the Vegetation on Danish Commons. Munksgaard in Komm. https://books.google.ca/books?id=rpS8GAAACAAJ.
37. **Anderson M.** 2001. A new method for non-parametric multivariate analysis of variance. *Austral Ecol* **26**:32–46.
38. **Lahti L, Shetty S.** 2023. microbiome: Microbiome Analytics (1.24.0). Bioconductor version: Release (3.18).
39. **Yan L.** 2023. ggvenn: Draw Venn Diagram by "ggplot2" (0.1.10).
40. **Neuwirth E.** 2022. RColorBrewer: ColorBrewer Palettes (1.1-3).
41. **Wickham H.** 2007. Reshaping Data with the reshape Package. *J Stat Softw* **21**:1–20.
42. **Xie Y.** 2023. knitr: A General-Purpose Package for Dynamic Report Generation in R (1.45).
43. **Cáceres MD, Legendre P.** 2009. Associations between species and groups of sites: indices and statistical inference. *Ecology* **90**:3566–3574.
44. **Love MI, Huber W, Anders S.** 2014. Moderated estimation of fold change and dispersion for RNA-seq data with DESeq2. *Genome Biol* **15**:550.
45. **Douglas GM, Maffei VJ, Zaneveld JR, Yurgel SN, Brown JR, Taylor CM, Huttenhower C, Langille MGI.** 2020. PICRUSt2 for prediction of metagenome functions. 6. *Nat Biotechnol* **38**:685–688.
46. **Wickham H, Hester J, Bryan J.** 2023. readr: Read Rectangular Text Data.
47. **Müller K, Wickham H.** 2023. tibble: Simple Data Frames.
48. **Dawson C.** 2022. ggprism: A "ggplot2" Extension Inspired by "GraphPad Prism."
49. **Pedersen T.** 2023. patchwork: The Composer of Plots.
50. **van den Brand T.** 2023. Hacks for "ggplot2" (0.2.6).
51. **Wickham H, François R, Henry L, Müller K.** 2022. dplyr: A Grammar of Data Manipulation.
52. **Calalang J, Cheung H, Lichimo K, So B.** 2021. Identifying breed, dietary, and reproductive factors affecting the gut microbiome of dogs with inflammatory bowel disease. *Undergrad J Exp Microbiol Immunol* **26**.
53. **Gong D, Gong X, Wang L, Yu X, Dong Q.** 2016. Involvement of Reduced Microbial Diversity in Inflammatory Bowel Disease. *Gastroenterol Res Pract* **2016**:6951091.
54. **Díaz-Regañón D, García-Sancho M, Villaescusa A, Sainz Á, Agulla B, Reyes-Prieto M, Rodríguez-Bertos A, Rodríguez-Franco F.** 2023. Characterization of the Fecal and Mucosa-Associated Microbiota in Dogs with Chronic Inflammatory Enteropathy. *Anim Open Access J MDPI* **13**:326.
55. **Kanno M, Katayama T, Morita N, Tamaki H, Hanada S, Kamagata Y.** 2015. Catenisphaera adipataccumulans gen. nov., sp. nov., a member of the family Erysipelotrichaceae isolated from an anaerobic digester. *Int J Syst Evol Microbiol* **65**:805–810.
56. **Kozhieva M, Naumova N, Alikina T, Boyko A, Vlassov V, Kabilov MR.** 2021. The Core of Gut Life: Firmicutes Profile in Patients with Relapsing-Remitting Multiple Sclerosis. *Life* **11**:55.

57. **Wang L, Liao Y, Yang R, Zhu Z, Zhang L, Wu Z, Sun X.** 2021. An engineered probiotic secreting Sj16 ameliorates colitis via Ruminococcaceae/butyrate/retinoic acid axis. *Bioeng Transl Med* **6**:e10219.
58. **Lopez-Siles M, Duncan SH, Garcia-Gil LJ, Martinez-Medina M.** 2017. Faecalibacterium prausnitzii: from microbiology to diagnostics and prognostics. *ISME J* **11**:841–852.
59. **Martín R, Rios-Covian D, Huillet E, Auger S, Khazaal S, Bermúdez-Humarán LG, Sokol H, Chatel J-M, Langella P.** 2023. Faecalibacterium: a bacterial genus with promising human health applications. *FEMS Microbiol Rev* **47**:fuad039.
60. **Lee AA, Rao K, Limsrivilai J, Gillilland M, Malamet B, Briggs E, Young VB, Higgins PDR.** 2020. Temporal Gut Microbial Changes Predict Recurrent Clostridiodes Difficile Infection in Patients With and Without Ulcerative Colitis. *Inflamm Bowel Dis* **26**:1748–1758.
61. **Kushkevych I, Dordević D, Kollár P.** 2019. Analysis of physiological parameters of Desulfovibrio strains from individuals with colitis. *Open Life Sci* **13**:481–488.
62. **Kaakoush NO.** 2020. Sutterella Species, IgA-degrading Bacteria in Ulcerative Colitis. *Trends Microbiol* **28**:519–522.
63. **Trakman GL, Fehily S, Basnayake C, Hamilton AL, Russell E, Wilson-O'Brien A, Kamm MA.** 2022. Diet and gut microbiome in gastrointestinal disease. *J Gastroenterol Hepatol* **37**:237–245.
64. **Singh RK, Chang H-W, Yan D, Lee KM, Ucmak D, Wong K, Abrouk M, Farahnik B, Nakamura M, Zhu TH, Bhutani T, Liao W.** 2017. Influence of diet on the gut microbiome and implications for human health. *J Transl Med* **15**:73.
65. **Ananthakrishnan AN, Luo C, Yajnik V, Khalili H, Garber JJ, Stevens BW, Cleland T, Xavier RJ.** 2017. Gut Microbiome Function Predicts Response to Anti-integrin Biologic Therapy in Inflammatory Bowel Diseases. *Cell Host Microbe* **21**:603-610.e3.
66. **Ramasundara M, Leach ST, Lemberg DA, Day AS.** 2009. Defensins and inflammation: The role of defensins in inflammatory bowel disease. *J Gastroenterol Hepatol* **24**:202–208.
67. **Papada E, Amerikanou C, Gioxari A, Kalogeropoulos N, Kaliora AC.** 2020. The Association of Plasma-Free Branched-Chain Amino Acids with Disease Related Parameters in Ulcerative Colitis. *Diagn Basel Switz* **10**.
68. **Hong S-KS, Maltz BE, Coburn LA, Slaughter JC, Chaturvedi R, Schwartz DA, Wilson KT.** 2010. Increased serum levels of L-arginine in ulcerative colitis and correlation with disease severity. *Inflamm Bowel Dis* **16**:105–111.
69. **Scoville EA, Allaman MM, Brown CT, Motley AK, Horst SN, Williams CS, Koyama T, Zhao Z, Adams DW, Beaulieu DB, Schwartz DA, Wilson KT, Coburn LA.** 2018. Alterations in Lipid, Amino Acid, and Energy Metabolism Distinguish Crohn's Disease from Ulcerative Colitis and Control Subjects by Serum Metabolomic Profiling. *Metabolomics Off J Metabolomic Soc* **14**:17.
70. **Koutroumpakis E, Ramos-Rivers C, Regueiro M, Hashash JG, Barrie A, Swoger J, Baidoo L, Schwartz M, Dunn MA, Koutroubakis IE, Binion DG.** 2016. Association Between Long-Term Lipid Profiles and Disease Severity in a Large Cohort of Patients with Inflammatory Bowel Disease. *Dig Dis Sci* **61**:865–871.
71. **Tefas C, Ciobanu L, Tanțău M, Moraru C, Socaciu C.** 2020. The potential of metabolic and lipid profiling in inflammatory bowel diseases: A pilot study. *Bosn J Basic Med Sci* **20**:262–270.
72. **McLaren MR, Willis AD, Callahan BJ.** 2019. Consistent and correctable bias in metagenomic sequencing experiments. *eLife* **8**:e46923.
73. **Bhang E, Rao A, Robinson A.** 2021. A potential age-dependent effect of antibiotics on the gut microbiome in dogs with inflammatory bowel disease. *Undergrad J Exp Microbiol Immunol* **7**.
74. **You I, Kim MJ.** 2021. Comparison of Gut Microbiota of 96 Healthy Dogs by Individual Traits: Breed, Age, and Body Condition Score. *Anim Open Access J MDPI* **11**.
75. **Reddy KE, Kim H-R, Jeong JY, So K-M, Lee S, Ji SY, Kim M, Lee H-J, Lee S, Kim K-H, Kim M.** 2019. Impact of Breed on the Fecal Microbiome of Dogs under the Same Dietary Condition. *J Microbiol Biotechnol* **29**:1947–1956.