Original Article

The alterations of fecal microbiota in dogs with acute diarrhea, Thailand

Jeerawat Soonthornsit[1*] Natharin Ngamwongsatit[2] Panpanga Sangsuriya[1] Nlin Arya[1]

Abstract

Acute diarrhea is a common clinical sign in dogs which the pathogenesis has associated with altered gut microbiome compositions and influenced by diet, environment, and medication. However, the impact of gut microbiome on dog health has still not been well elucidated in Thailand. This study aims to investigate the alterations of the fecal microbiome in dogs with diarrhea (<3 days) compared to healthy dogs. Fresh feces were collected once from each dog, and DNA extraction was performed following the QIAamp DNA stool mini kit protocol. The *16s rRNA* gene, then, amplified and analyzed by the next-generation DNA sequencing technology. The results showed a significant reduction of the evenness and Shannon index in dogs with the diarrhea group. The most dominant phylum in healthy dogs, Firmicutes, was markedly declined. Additionally, significant decreases in bacterial abundances were observed in twelve genera. To the best of our knowledge, seven of them were first described in their alterations including *Clostridium sensu stricto 1*, *Lachnospiraceae NKA4136 group*, *Erysipelatoclostridium*, *Tyzzerella* 3, *Alloprevotella* and *Slackia*. Therefore, these findings disclose the new possible biomarkers for intestinal diseases and the effect of ordinary diets on the fecal microbiome in dog health, Thailand.

Keywords: dogs, acute diarrhea, healthy, microbiome, Thailand

[1]*Department of Pre-clinic and Applied Animal Science, Faculty of Veterinary Science, Mahidol University, 999 Phutthamonthon 4 Road, Salaya, Phutthamonthon Nakhonpathom, 73170, THAILAND*

[2]*Department of Clinical Sciences and Public Health, Faculty of Veterinary Science, Mahidol University, 999 Phutthamonthon 4 Road, Salaya, Phutthamonthon Nakhonpathom, 73170, THAILAND*

****Correspondence**: jeerawat.soo@mahidol.edu* (J. Soonthornsit)

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Introduction

Microbiome, a term that describes the genome of all the microorganisms, has garnered strong interest due to the potential role in host health and disease (Barko *et al*., 2018). The characterization of the microbiome can be investigated by molecular-phylogenetic studies, based on *16S rDNA* gene analysis. Recent advanced technology, whole-genome shotgun sequencing has been employed to investigate microbial gene repertoires and identified numerous microbes, and revealed uncultured genera. The combination of sequencing technology and computational tools reveals valuable data for the microbiome in humans and animals (Swenson *et al*., 2011; Garcia-Mazcorro *et al*., 2013).

Altered compositions in microbiota associated with diseases or conditions that change microbe-host homeostasis are called dysbiosis, which is characterized by a reduction in microbial species diversity and a change of microbial communities (Barko *et al*., 2018; Staley *et al*., 2018). Dysbiosis-associated gastrointestinal diseases in dogs have been reported especially in inflammatory bowel disease (IBD), chronic enteropathies, non-hemorrhagic, and acute hemorrhagic diarrhea (Suchodolski *et al*., 2012b; Honneffer *et al*., 2014). Acute diarrhea is a common clinical sign in dogs, which can be resolved by spontaneous, symptomatic, or specific treatments depending on the causes of diarrhea such as endoparasite, dietary indiscretion, bacterial enteritis, toxin, etc. There has been evidence that the changes in the gastrointestinal (GI) microbiome play a crucial role in acute and chronic enteropathy (Allenspach *et al*., 2010; Barko *et al*., 2018; Pilla and Suchodolski, 2020). The significant alterations of microbial communities were also detected. Bacteroidetes and Firmicutes, especially genus *Faecalibacterium* and *Ruminococcaceae*, were decreased, while *Clostridium* has been overrepresented (Guard *et al*., 2015).

Although the GI microbiome in acute diarrhea has been reported in dogs, most of the studies were explored in a Western country (Suchodolski *et al*., 2012b; Guard *et al*., 2015; Unterer and Busch, 2021). There was a study in humans that geography and cultural traditions influenced to features of gut microbiomes (Yatsunenko *et al*., 2012). Therefore, this study aimed at characterizing fecal microbiome in dogs with acute diarrhea compared to a healthy dog in Thailand using whole-genome shotgun sequencing, which has empowered the taxonomic analysis. Additionally, the influencing factors associated with acute diarrhea, including diet and water, were also evaluated.

Materials and Methods

Ethics Statement: This study was approved by the Faculty of Veterinary Science, Mahidol University-Institute Animal Care and Use Committee (FVS-MU-IACUC) (approval number: MUVS-2017-10-49).

Animals: Healthy dogs (n=29) and dogs with acute diarrhea (n=11), older than 1 year were chosen for this experiment. All dogs were privately owned, lived in home environments with fences, and obtained complete health programs such as vaccination and deworming. The control group consisted of healthy dogs, that free from the clinically apparent disease within a month. In the diarrheal group, all dogs presented with the duration of diarrhea $\leq$3 days and the absence of other concurrent diseases. Dogs with internal parasitic and viral infections were excluded from this experiment. Dogs were grouped by the types of food (commercial, home-cooked, and mixed diet) and source of water (drinking and tap water). Rectal contents were obtained once from dogs and then the routine fecal examination was performed for ruling out endoparasite infection. All remaining samples were kept immediately at a -20°C, until DNA extraction.

DNA extraction: Genomic DNA was extracted using the QIAamp DNA Stool Mini Kit protocol (Qiagen, USA). DNA concentrations of each sample were estimated by spectrophotometry, NanoDrop One (Thermo Fisher Scientific Inc., Madison, WI, USA). The extracted DNA samples were pooled, as mentioned above, then stored at -20°C until 16S metagenomic sequencing.

16S amplicon library preparation and sequencing: *16S rRNA* gene was amplified from metagenomic DNA samples using primer targeting V3-V4 region. The amplification condition included an initial denaturation step 3 min at 94°C, followed by 25 cycles of 98°C for 20 sec, 55°C for 30 sec, and 72°C for 30 sec, followed by a single step final extension step at 72°C for 5 min. Subsequently, the purified 16S amplicon was indexed using a 2X KAPA hot-start ready mix and 5 µl of each Nextera XT index primer in a 50 µl PCR reaction, followed by 8-10 cycles of PCR condition. We used AMPure XP beads for cleaning PCR products in every step. Finally, the indexed 16S amplicon was pooled and diluted to the final loading concentration at 6 pM. Cluster generation and 250-bp paired-end read sequencing were performed on an Illumina MiSeq at Omics Sciences and Bioinformatics Center (Chulalongkorn University, Bangkok, Thailand).

Bioinformatics: FATSQ raw data were generated and demultiplexed using Miseq reporter software v3.1. Targeted V3-V4 primer sequences were removed, and the data was imported to QIIME2 software (v2019.7). Denoised reads were clustered into amplicon sequence variants (equivalent to observed operational taxonomic units, OTUs). Then, a phylogenetic tree was built using SEPP QIIME 2 plugin. Ten thousand sequencing reads were used for rarefying.

Statistical analysis: Observed OTUs, Faith's PD, and Shannon's diversity index were measured. Kruskal-Wallis statistical test was performed for alpha diversity group significance (P-value <0.05). We assigned taxonomy to the OTUs using a Naive-Bayes approach implemented in the scikit learn Python library and the SILVA database, and classification stacked bar plots were created in the Phylum level. Identifying statistically differentially abundant taxa was performed using white's non-parametric t-test implemented in STAMP (Statistical Analysis of Taxonomic and Functional Profiles) software. The

observed frequencies of different bacterial genera in healthy and diarrheal groups were calculated and presented in the percentages. The relationships between different genus frequencies in each group and management parameters were analyzed using the Pearson's Chi-squared test.

Results

Sequence analysis: Ten thousand sequencing reads were used for rarefying. Rarefaction curve, a representation of the species richness (number of different species) for a given number of individual samples, had plateaued indicating that complete sampling of these environments had been sufficient for this experiment (Figs. 1).

Diversity analysis: The analysis of alpha diversity, the average species diversity in each sample, showed that the reduction of evenness (species-abundance distributions) and Shannon index (species diversity) were statistically significantly detected in the diversity of dogs with acute diarrhea when compared to healthy dogs (P<0.05) (Fig. 1A-1B). In this study, OTUs represented a taxonomic unit of a bacterial genus. OTUs and Faith's phylogenetic diversity (Faith's PD) also tended to reduce in a diarrheal group, however, there is no significant difference in the diversity between those groups (P>0.05) (Fig. 1C-1D).

Fecal bacterial compositions in healthy dog and dogs with acute diarrhea: The Taxonomic bars represent the bacterial composition in microbiota at taxonomic levels. In phylum classification, Firmicutes and Bacteroidetes were predominant in healthy dogs. However, the microbiome in dogs with acute diarrhea showed variables in the abundance of Firmicutes and Proteobacteria (Fig. 2). Significant decreases in proportions of the diarrheal group were observed in twelve genera including *Faecalibacterium, Ruminococcus, Blautia, Lachnospira, Lachnospiraceae NKA4136 group, Erysipelatoclostridium, Clostridium sensu stricto 1, Tyzzerella 3* belonging to the phylum Firmicutes; *Helicobacter* belonging to the phylum Proteobacteria; *Slackia* belonging to the phylum Actinobacteria; and *Alloprevotella, Prevotella* belonging to the phylum Bacteroidetes (P<0.05) (Fig. 3). The alteration of prominent members of the microbiota, *Faecalibacterium, Blautia, Clostridium sensu stricto, Alloprevotella,* and *Prevotella* was displayed using a heatmap (Fig. 4).

Relationship between bacterial diversity and management parameters: The relationship between bacterial diversity and management parameters revealed that the diet significantly influenced the alteration of the microbiome (P<0.05). Both healthy and acute diarrheal dogs fed with food combining diets had higher frequencies of observed bacterial genera in the feces compared to commercial diets only. Water management was not related to the change of bacterial diversity between both groups in this experiment (P>0.05) (Table 1).

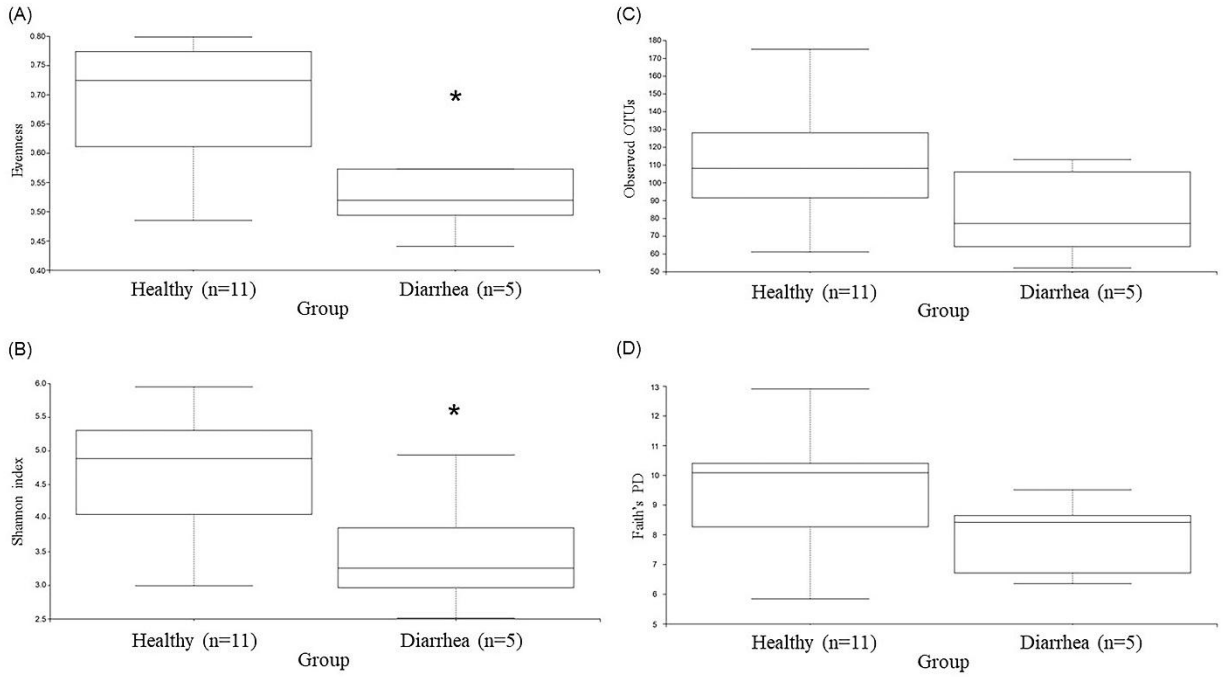

Figure 1 Evenness (A), Shannon index (B), observed OTUs (C) and Faith's PD box (D) plots of healthy group and dogs with diarrheic group, * P<0.05.

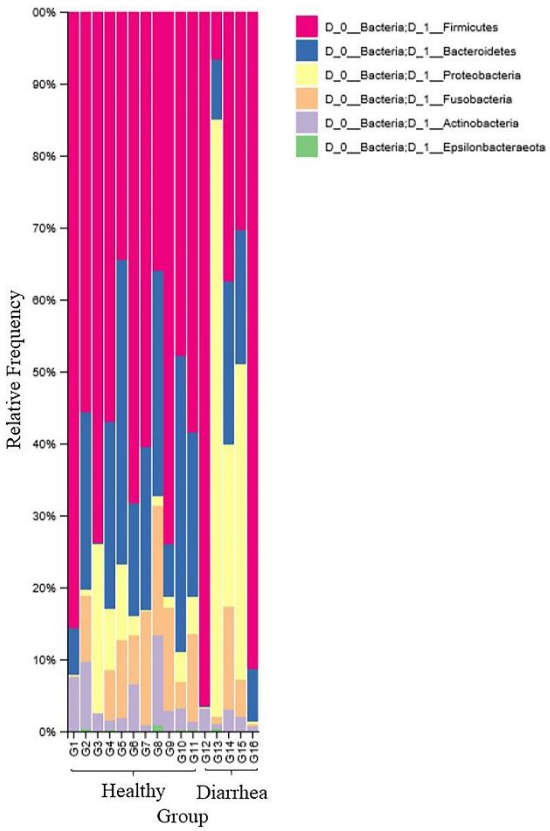

Figure 2 Phylum-level compositions and comparison of bacterial flora in all fecal samples of healthy and diarrheal dogs.

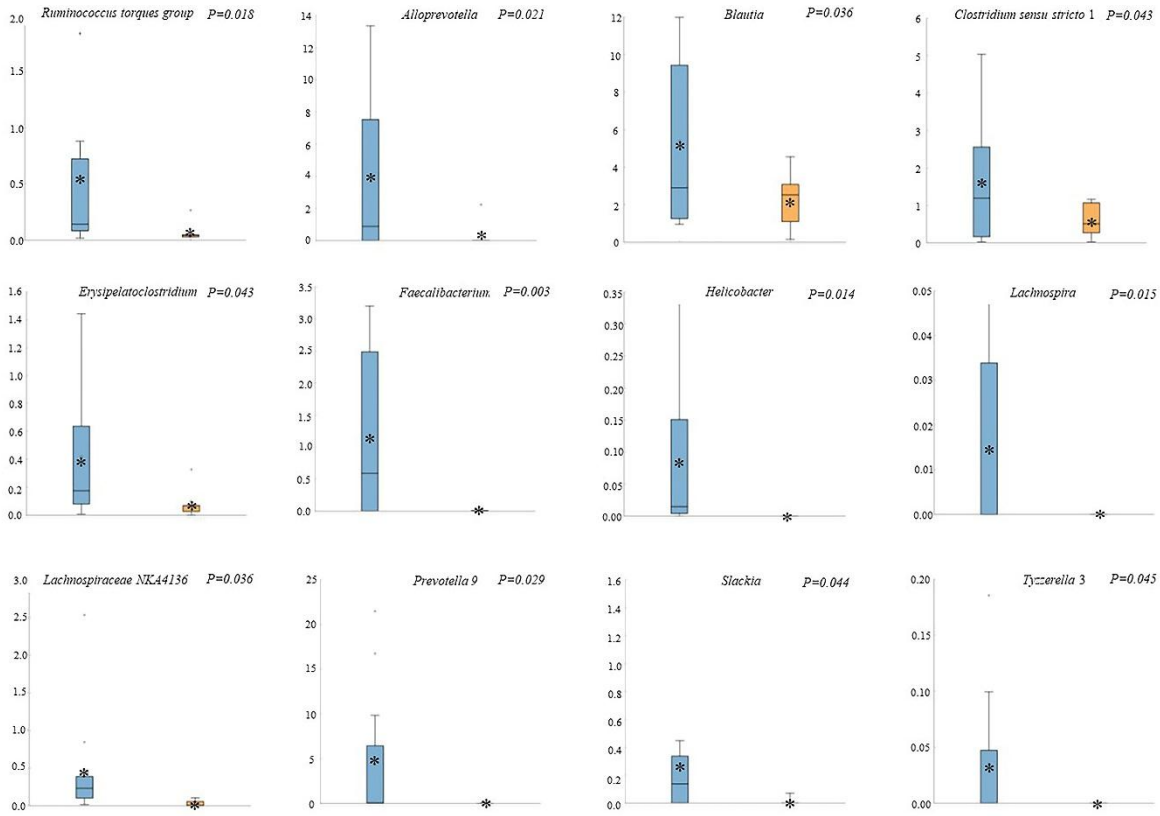

Figure 3 Box plots of % relative abundance of Faecalibacterium, Lachnospira, Lachnospiraceae NKA4136 group, Ruminococcus, Blautia, Erysipelatoclostridium, Clostridium sensu stricto 1, Tyzzerella 3, Helicobacter, Slackia, Alloprevotella and Prevotella in control and treatment groups. * P<0.05.

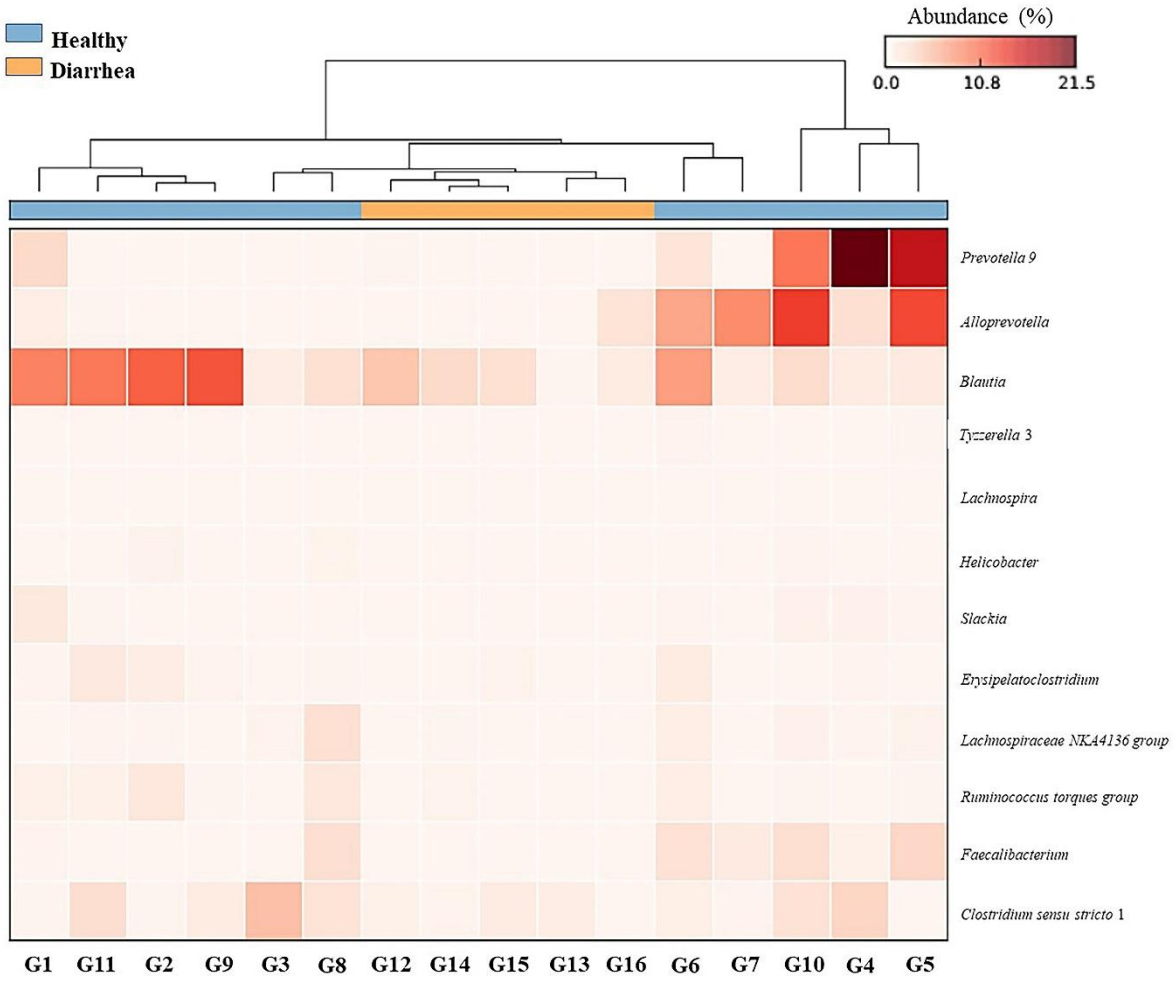

Figure 4 Heatmap illustrating the relative abundance of prominent bacterial genera in fecal samples of healthy and diarrheal groups.

Table 1 Relationship between management parameters and observed frequencies of different bacterial genera of fecal samples collected from healthy and diarrheal groups.

Management parameters		*Animal groups*		*P*-value
		Healthy (N=1,213)	*Acute diarrhea* (N = 412)	
		n (%)	n (%)	
Diet	Commercial diet	200 (16.5%)	141 (34.2%)	<0.05
	Commercial and homed-cook diet	1,013 (83.5%)	271 (65.8%)	
Water source	Tap water	807 (71.7%)	296 (71.8%)	>0.05
	Filtered water	343 (28.3%)	116 (28.2%)	

Pearson's Chi-Square test

Discussion

The gut microbiome in dogs has been identified that alterations of bacterial abundance were found during acute diarrhea. These alterations had affected the production of short-chain fatty acids (SCFAs), which were essential for gut host health. The main SCFA-producing bacteria have belonged to the members of phylum Firmicutes such as *Faecalibacterium*, *Blautia*, *Ruminococcus*, and *Turicibacter* (Pilla and Suchodolski, 2020). In this study, dogs with acute diarrhea showed a reduction in Shannon index and evenness compared to healthy dogs. The relative abundance of bacteria phyla showed profound alteration in Firmicutes and Proteobacteria which more than half of the samples exhibited trends for lower Firmicutes and higher Proteobacteria abundances. While Firmicutes, Bacteriodetes, and Proteobacteria were the first, second, and third most dominant phyla in healthy dogs, respectively, which agree to previous reports (Suchodolski, *et al.*, 2008; Handl *et al.*, 2011; Coelho *et al.*, 2018).

In dogs with acute diarrhea, we found that the significant reduction in abundances of twelve genera, including *Faecalibacterium, Lachnospira, Lachnospiraceae NKA4136 group, Ruminocoocus, Blautia,*

Erysipelatoclostridium, Clostridium sensu stricto, Tyzzerella 3, *Helicobacter, Slackia, Alloprevotella*, and *Prevolella*, most of them were the members of phylum Firmicutes. The alterations of those bacteria in twelve genera indicated the GI dysbiosis in dogs with diarrhea which possibly affected the production of essential nutrients, dietary breakdown, and immune system. Then, the essential roles of the twelve genera in dogs' health should be further examined.

The members of phylum Firmicutes play important roles to support the health of intestinal villi by SCFAs including acetate, propionate, and butyrate, which are an essential energy source for colonocytes. Moreover, they help to promote cell division, mucin production, antimicrobial peptide, and anti-inflammatory compounds secretion, strengthening tight junctions and intestinal motility (Barko *et al.,* 2018, Pilla and Suchodolski, 2020). In this study, we demonstrated that dogs with acute diarrhea had a significant reduction in abundances of prominent genera of Firmicutes, including *Faecalibacterium, Ruminococcus*, and *Blautia*. *Faecalibacterium* has been identified as a butyrate-producing bacteria. Loss in these bacterial abundances, as well as *Ruminococcus*, were also significantly correlated with the concentrations of fecal propionic acid in dogs with acute diarrhea (Guard *et al.,* 2015; Pilla and Suchodolski, 2020). The genus *Blautia* is involved in glucose metabolism producing the major end product such as acetate, ethanol, hydrogen, lactate, and succinate (Liu *et al.,* 2008). The decrease of *Blautia* has been identified in *Clostridium difficile* infection in humans and *C. perfringens* toxin-associated hemorrhagic diarrhea in dogs (Antharam *et al.,* 2013; Ziese *et al.,* 2018).

In addition to the members of Firmicutes, *Clostridium sensu stricto 1* also decreased in the acute diarrhea group, although it was found that enriched in chronic enteropathy (CE) and large bowel diarrhea/colitis in dogs (Wang *et al.,* 2019). Moreover, the marked depletion of *Lachnospira, Lachnospiraceae NKA4136 group, Erysipelatoclostridium*, and *Tyzzerella* 3, were also noticed. *Lachnospira* and *Lachnospiraceae NKA4136 group* have been reported as normal inhabitants of the gastrointestinal tracts (Cottaand Forster, 2006; Hu *et al.,* 2019; Wang *et al.,* 2017). *Erysipelatoclostridium* involves the metabolism of proteins and saccharides producing acetate and lactate (Oliphant and Allen-Vercoe, 2019). It has been identified as possible biomarkers for major intestinal diseases such as Crohn's disease and *Clostridium difficile* infection in humans (Mancabelli *et al.,* 2017). The relative abundance of *Tyzzerella* has been reported that rose in patients with ulcerative colitis and irritable bowel syndrome (Agnello *et al.,* 2017; Qiu, *et al.,* 2017). In animals, *Tyzzerella* 3 is a common genus in red swamp crayfish (*Procambarus clarkia*) and female prairie vole (*Microtus ochrogaster*) (Curtis *et al.,* 2018; Shui *et al.,* 2020), while the presence of *Tyzzerella* 3 had not been described in dogs.

Phylum Proteobacteria is commonly colonized in the small intestine and presents a smaller number in the fecal sample. The members of this phylum, such as *Escherichia*, are associated with diseases including chronic enteropathy (Pilla and Suchodolski, 2020). However, the alteration of *Escherichia* was not found in this study. On the other hand, we recognized the significant reduction of *Helicobacter* which this alteration had not been well described in dogs with acute diarrhea so far. In dogs with chronic diarrhea, *H. heilmannii* is a commonly occurring species in the canine fecal sample (Jankowski *et al.,* 2016). This species supposed to increase in the frequency of chronic diarrhea and involve more severe gastritis (Kubota-Aizawa *et al.,* 2017).

In addition to the altered microbiome in a dog with acute diarrhea, the genera *Alloprevotella* and *Prevotella* belonging to phylum Bacteroidetes, and *Slackia* spp. belonging to phylum Actinobacteria were also markedly decreased. *Alloprevotella* and *Prevotella* have associated with the host's health. Their relative abundance significantly decreased in the acute dog with acute diarrhea as reported by previous studies (Guard *et al.,* 2015, Wang *et al.,* 2019). *Slackia* spp. have been thought to be important to gut health because of a strong correlation with the fecal score of cats with naturally occurring chronic diarrhea after being fed a therapeutic diet (Ramadan *et al.,* 2014).

The factors, that have influenced the alterations of microbiome composition, are diet, environment, and medication (Barko *et al.,* 2018). A history of scavenging or change of diet in the previous week has been associated with an increased risk of diarrhea in dogs (Stavisky *et al.,* 2011). Additionally, feeding a raw diet had a higher risk of microorganism contamination such as *Escherichia coli, Salmonella* spp., *Campylobacter* spp., *Listeria monocytogenes, Yersinia enterocolitica, Brucella* spp., *Staphylococcus aureus, and Clostridium* spp. compared to a heat-treated diet (Davies *et al.,* 2019). In this study, the home-cooked diet seemed to decrease the risks of diarrhea in dogs. Their reason might be that all home-cooked recipes were treated by heat and made with ordinary foodstuff, not raw or scavenging foods. Moreover, a commercial diet, has less palatability, that dog may not finish it at once, and leaving food out too long in a tropical climate, microorganisms grow rapidly in the range of warm temperatures causing food spoilage increasing the risks of diarrhea (Hammond *et al.,* 2015).

The limitation to this study is the small number of animals that were enrolled in the disease group. Additionally, the samples were pooled and grouped by types of food and source of drinking water, due to the limited amount of sample for whole-genome shotgun sequencing. Therefore, only the estimations of bacterial diversities were measured. Moreover, no sample was collected from dogs that were fed with a home-cooked diet alone. Hence, the evaluation of dietary influence including a home-cooked diet alone should be further investigated.

In conclusion, the results of this study demonstrated the characteristics of the fecal microbiome in dogs with acute diarrhea compared to healthy dogs in central Thailand. The significant reductions in abundances of twelve genera were identified in dogs with acute diarrhea. Some of them, including *Clostridium sensu stricto* 1, *Lachnospiraceae NKA4136 group, Erysipelatoclostridium, Tyzzerella* 3, *Alloprevotella* and *Slackia,* were first described in their alterations. Furthermore, the evaluation of management parameters revealed that a heated home-

cooked diet combined with a commercial diet had low risks of diarrhea in this study. Therefore, these findings disclose the new possible biomarkers for acute diarrheal diseases and the effect of ordinary diets on the fecal microbiome in dog health, Thailand. Factors that play important role in this alteration may include the environment and humidity that suitable for some bacteria biology, type of diet, and genetic of the individual. Finally, it is important to conduct more studies to elucidate the role of each factor. These pieces of knowledge will benefit the health and wellbeing of a dog's life.

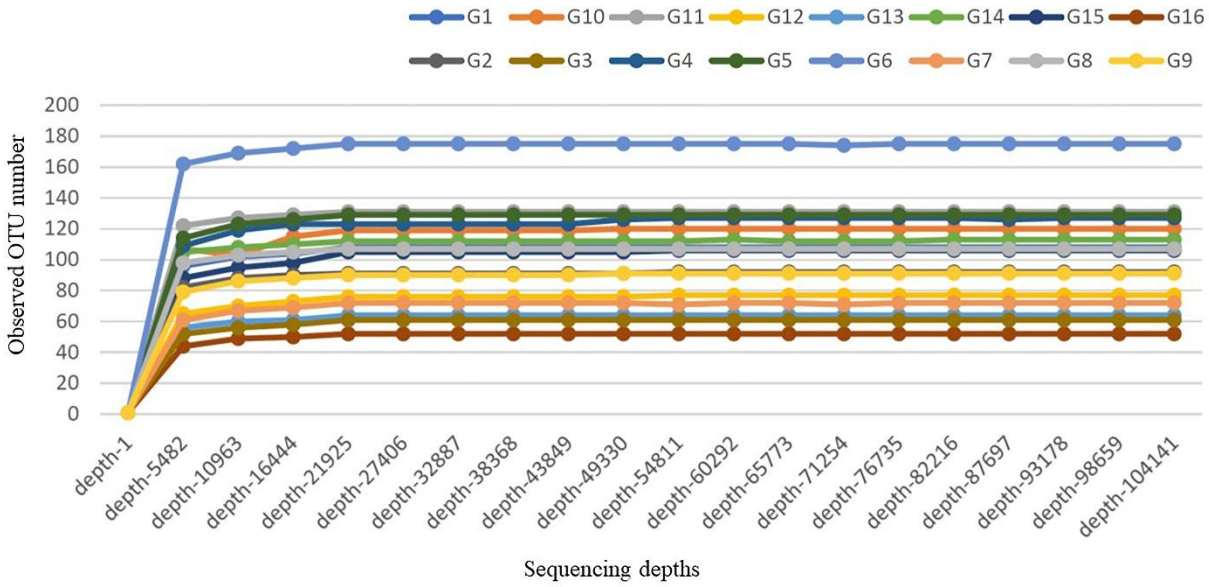

Figure Supplement 1 Rarefaction analysis of 16 S rRNA gene sequences obtained from canine fecal samples. Lines represent the species richness (microbial community) for a given number of individual samples. G1-G11: Samples from healthy dogs, G12-G16: Sample from dogs with diarrhea.

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References

Agnello M, Carroll LN, Imam N, Pino R, Palmer C, Varas I, Greene C, Hitschfeld M, Gupta S, Almonacid DE and Hoaglin MC 2020. Gut microbiome composition and risk factors in a large cross-sectional IBS cohort. BMJ Open Gastroenterol. 7: e000345.

Allenspach K, House A, Smith, K, McNeill FM, Hendricks A, Elson-Riggins J, Riddle A, Steiner JM, Werling D, Garden OA, Catchpole B and Suchodolski JS 2010. Evaluation of mucosal bacteria and histopathology, clinical disease activity and expression of Toll-like receptors in German shepherd dogs with chronic enteropathies. Vet Microbiol. 146: 326-35.

Antharam VC, Li EC, Ishmael A, Sharma A, Mai V, Rand KH and Wang GP 2013. Intestinal dysbiosis and depletion of butyrogenic bacteria in *Clostridium difficile* infection and nosocomial diarrhea. J Clin Microbiol. 51: 2884-2892.

Barko PC, McMichael MA, Swanson KS and Williams DA 2018. The gastrointestinal microbiome: A Review. J Vet Intern Med. 32: 9-25.

Coelho LP, Kultima JR, Costea PI, Fournier C, Pan Y, Czarnecki-Maulden G, Hayward MR, Forslund SK, Schmidt T, Descombes P, Jackson JR, Li Q and Bork P 2018. The similarity of the dog and human gut microbiomes in gene content and response to diet. Microbiome. 6: 72.

Cotta M and Forster R 2006. The family Lachnospiraceae, including the genera *Butyrivibrio, Lachnospira*, and *Roseburia*. Prokaryotes. 4: 1002–1021.

Curtis JT, Assefa S, Francis A and Köhler GA 2018. Fecal microbiota in the female prairie vole (*Microtus ochrogaster*). PLOS ONE. 13: e0190648.

Davies RH, Lawes JR and Wales AD 2019. Raw diets for dogs and cats: a review, with particular reference to microbiological hazards. J Small Anim Pract. 60: 329-339.

Garcia-Mazcorro JF and Minamoto Y 2013. Gastrointestinal microorganisms in cats and dogs: a brief review. Arch Med Vet. 45: 111-24.

Guard BC, Barr JW, Reddivari L, Klemashevich C, Jayaraman A, Steiner JM, Vanamala J and Suchodolski JS 2015. Characterization of microbial dysbiosis and metabolomic changes in dogs with acute diarrhea. PLOS ONE. 10: e0127259.

Hammond ST, Brown JH, Burger JR, Flanagan TP, Fristoe TS, Mercado-Silva N, Nekola JC, and Okie JG 2015. Food Spoilage, Storage, and Transport: Implications for a Sustainable Future, BioScience. 65: 758–768.

Handl S, Dowd SE, Garcia-Mazcorro JF, Steineri JM and Suchodolski JS 2011. Massive parallel 16S rRNA gene pyrosequencing reveals highly diverse fecal bacterial and fungal communities in healthy dogs and cats. FEMS Microbiol Ecol. 76:301-310.

Honneffer JB, Minamoto Y and Suchodolski JS 2014. Microbiota alterations in acute and chronic

gastrointestinal inflammation of cats and dogs. World J Gastroenterol. 20: 16489-97.

Hu S, Wang, J, Xu Y, Yang H, Wang J, Xue C, Yan X and Su L 2019. Anti-inflammation effects of fucosylated chondroitin sulfate from *Acaudina molpadioides* by altering gut microbiota in obese mice. FOOD FUNCT. 10: 1736-1746.

Jankowski M, Spużak J, Kubiak K, Glińska-Suchocka K and Biernat M 2016. Detection of gastric *Helicobacter* spp. in stool samples of dogs with gastritis. Pol J Vet Sci. 19: 237-243.

Kubota-Aizawa S, Ohno K, Fukushima K, Kanemoto H, Nakashima K, Uchida K, Chambers JK, Goto-Koshino Y, Watanabe T, Sekizaki T, Mimuro H and Tsujimoto H 2017. Epidemiological study of gastric *Helicobacter* spp. in dogs with gastrointestinal disease in Japan and diversity of *Helicobacter heilmannii sensu stricto*. Vet J. 225: 56-62.

Liu C, Finegold SM, Song Y and Lawson PA 2008. Reclassification of *Clostridium coccoides, Ruminococcus hansenii, Ruminococcus hydrogenotrophicus, Ruminococcus luti, Ruminococcus productus, and Ruminococcus schinkii* as *Blautia coccoides* gen. nov., comb. nov., *Blautia hansenii* comb. nov., *Blautia hydrogenotrophica comb. nov.*, Blautia luti comb. nov., Blautia producta comb. nov., Blautia schinkii comb. nov. and description of *Blautia wexlerae* sp. nov., isolated from human feces. Int J Syst Evol Microbiol. 58: 189-902.

Mancabelli, L, Milani C, Lugli GA, Turroni F, Cocconi D, van Sinderen D and Ventura M 2017. Identification of universal gut microbial biomarkers of common human intestinal diseases by meta-analysis. FEMS Microbiol Ecol. 93: 10.1093/femsec/fix153.

Oliphant K and Allen-Vercoe E 2019. Macronutrient metabolism by the human gut microbiome: major fermentation by-products and their impact on host health. Microbiome. 7: 91.

Pilla R and Suchodolski JS 2020. The role of the canine gut microbiome and metabolome in health and gastrointestinal disease. Front Vet Sci. 6: 498.

Qiu Z, Yang H, Rong L, Ding W, Chen J and Zhong L 2017. Targeted metagenome-based analyses show gut microbial diversity of inflammatory bowel disease patients. Indian J Microbiol. 57: 307–315.

Ramadan Z, Xu H, Laflamme D, Czarnecki-Maulden G, Li Q J, Labuda J and Bourqui B 2014. The fecal microbiota of cats with naturally occurring chronic diarrhea assessed using 16S rRNA gene 454-pyrosequencing before and after dietary treatment. J Vet Intern Med. 28: 59-65.

Shui Y, Guan Z, Liu G and Fan L-M 2020. Gut microbiota of red swamp crayfish *Procambarus clarkii* in integrated crayfish-rice cultivation model. AMB Expr, 10: 5.

Staley C, Kaiser T, and Khoruts A 2018. Clinician Guide to Microbiome Testing. Dig Dis Sci. 63(12): 3167–3177.

Stavisky J, Radford AD, Gaskell R, Dawson S, German A, Parsons B, Clegg S, Newman J and Pinchbeck G 2011. A case-control study of pathogen and lifestyle risk factors for diarrhea in dogs. Prev Vet Med. 99: 185-192.

Suchodolski JS, Camacho J and Steiner JM 2008. Analysis of bacterial diversity in the canine duodenum, jejunum, ileum, and colon by comparative 16S rRNA gene analysis. FEMS Microbiol Ecol. 66: 567-78.

Suchodolski JS, Markel ME, Garcia-Mazcorro JF, Unterer S, Heilmann RM, Dowd SE, Kachroo P, Ivanov I, Minamoto Y, Dillman EM, Steiner JM. Cook AK and Toresson L 2012[b]. The fecal microbiome in dogs with acute diarrhea and idiopathic inflammatory bowel disease. PLOS ONE. 7: e51907.

Suchodolski JS, Xenoulis PG, Paddock CG, Steiner JM, and Jergens AE 2010. Molecular analysis of the bacterial microbiota in duodenal biopsies from dogs with idiopathic inflammatory bowel disease. Vet Microbiol. 142: 394-40.

Swanson KS, Dowd SE, Suchodolski JS, Middelbos IS, Vester BM, Barry, KA, Nelson, KE, Torralba M, Henrissat B, Coutinho PM, Cann IK, White BA and Fahey Jr GC 2011. Phylogenetic and gene-centric metagenomics of the canine intestinal microbiome reveals similarities with humans and mice. ISME J. 5: 639-649.

Unterer S and Busch K 2021. Acute hemorrhagic diarrhea syndrome in dogs. Vet Clin Small Anim. 5: 79–92.

Wang C, Huang Z, Yu K, Ding R, Ye K, Dai C, Xu X, Zhou G and Li C 2017. A high-salt diet has a certain impact on protein digestion and gut microbiota: a sequencing and proteome combined study. Front Microbiol. 8:1838.

Wang S, Martins R, Sullivan MC, Friedman ES, Misic AM, El-Fahmawi A, De Martinis E, O'Brien K, Chen Y, Bradley C, Zhang G, Berry A, Hunter CA, Baldassano RN, Rondeau MP and Beiting DP 2019. Diet-induced remission in chronic enteropathy is associated with altered microbial community structure and synthesis of secondary bile acids. Microbiome. 7: 126.

Yatsunenko T, Rey F, Manary M, Trehan I, Dominguez-Bello MG, Contreras M, Magris M, Hidalgo G, Baldassano RN, Anokhin AP, Heath AC, Warner B, Reeder J, Kuczynski J, Caporaso JG, Lozupone CA., Lauber C, Clemente JC, Knights D, Knight R and Gordon JI 2012. The human gut microbiome is viewed across age and geography. Nature. 486: 222–227.

Ziese AL, Suchodolski JS, Hartmann K, Busch K, Anderson A, Sarwar F, Sindern N and Unterer S 2018. Effect of probiotic treatment on the clinical course, intestinal microbiome, and toxigenic *Clostridium perfringens* in dogs with acute hemorrhagic diarrhea. PLOS ONE. 13: e0204691.