Inflammatory bowel disease (IBD) is a common cause of idiopathic, chronic, and relapsing gastrointestinal (GI) disease in dogs. The most typical histological change associated with IBD is lymphocytic-plasmacytic enteritis (LPE). The cause of symptomatic LPE is unknown, but it is believed to be secondary to the complex interplay between genetics, immune dysregulation, and environmental factors, including the GI microbiome. As for the treatment of the dogs with symptomatic LPE, a diet, an antimicrobial agent, a corticosteroid agent and an immunosuppressive drug are used frequently. However, some of the dogs with symptomatic LPE which is resistant to those treatments is present. Therefore, the establishment of the new treatment of dogs with symptomatic LPE is urgent business.

In human medicine, fecal microbiota transplantation (FMT) has been reported as an effective treatment for recurrent *Clostridium difficile* infections (CDI), irritable bowel syndrome and disease such as IBD. In FMT, fecal matter is collected from a tested healthy donor, mixed with saline or other solutions, strained, and administered to a patient by colonoscopy, endoscopy, sigmoidoscopy, or enema.

In veterinary medicine, FMT has recently been tested as a treatment option for multiple GI diseases, such as CDI and acute diarrhea. However, changes in microbiome diversity after treatment with FMT has not been reported for canine symptomatic LPE. Moreover, whether FMT is an effective and safe treatment for canine symptomatic LPE still remains unknown.

A purpose of this study is to consider being the treatment that FMT for the dogs with symptomatic LPE is safe and effective. Therefore, we pushed forward this study by the following contents by this thesis.

Chapter 2. Investigation of the microbiome in the feces in the symptomatic LPE dogs.

The classification of clinical symptoms used the dog inflammatory bowel disease activity index (CIBDAI) and Waltham Faeces Scoring system. CIBDAI of the symptomatic LPE dogs (sLPE) group was 13.5±1.4, and the asymptomatic LPE dogs (aLPE) group was 0.0±0.0. Waltham Faeces Scoring system of the sLPE group was 5.0±0.0, and the aLPE group was 2.0±0.0. CIBDAI and Waltham Faeces Scoring system of the sLPE group were significantly high level than aLPE group (respectively, $p < 0.01$). The comparison of the microbiome in the feces with both groups was carried out by 16S rRNA sequence analysis. In the sLPE group, α diversity of the microbiome significantly decreased as compared with aLPE group ($p < 0.03$). Also, the UniFrac distance for β diversity of the microbiome between both groups was the distance significantly away than the distance in the aLPE group ($p < 0.01$). Therefore we judged difference of the microbiome in both groups to be high. The predominant bacterial phylum was Proteobacteria (23.7-80.3 %) of total bacterial population in the sLPE group, and it was significantly higher than aLPE group (0.2-3.8 %) ($p < 0.01$). While Fusobacteria comprised < 0.1 % in the sLPE group, it was significantly lower than aLPE group (6.5-24.6 %) ($p < 0.01$). In the sLPE group, the phylum of Firmicutes and Bacteroidetes comprised 4.4-51.8 % and 0.1-24.5 % respectively, whereas in the aLPE group, Firmicutes and Bacteroidetes comprised 25.9-71.0 % and 7.9-45.0 % respectively. In the sLPE group, Firmicutes and Bacteroidetes were the low level of the thing which was not significant than the aLPE group. It was determined that the sLPE group was in a state of dysbiosis by the difference in these bacterial comprising. Also it was suggested that the dysbiosis was associated with clinical symptom.

Chapter 3. Examination about the variability of the microbiome and short-chain fatty acid (SCFA) in the feces in the aLPE dogs.

Except for a small portion of SCFA obtained directly from food, most are produced by intestinal microbial anaerobic fermentation. SCFA also improve gut health through several partial effects, including maintaining the integrity of the intestinal barrier, producing mucus and preventing inflammation. From the results of Chapter 2, Fusobacteria significantly decreased in the sLPE group than aLPE group. In the sLPE group, Firmicutes and Bacteroidetes were the low level of the thing which was not significant than the aLPE group. It was determined that the sLPE group was in a state of dysbiosis by the difference in these bacterial comprising. We hypothesized that the variability of these dominant bacterial species in healthy dogs affected of SCFA concentrations. It was investigated the variability of the fecal microbiome and concentrations of SCFAs for the aLPE dogs and explored the relationships between these parameters. Subsequently, the fecal microbiome was analyzed using quantitative PCR, whereas the concentration of SCFAs was quantified using gas chromatography. In this study, we used two type of probiotics to make the microbiome fluctuate artificially, which contains only one strain and three strains of beneficial bacteria during a period according to each. *Bacteroides* (coefficient estimate 2.46, $p < 0.01$), *Fusobacterium* (coefficient estimate 2.28, $p < 0.01$), *Ruminococcaceae* (coefficient estimate 2.07, $p = 0.04$) and *C. coccoides* group (coefficient estimate 1.42, $p < 0.01$) were positively correlated with acetic acid concentration, whereas *Bacteroides* (coefficient estimate 0.97, $p < 0.01$) and *Fusobacterium* (coefficient estimate 0.79, $p < 0.01$) were showed a positive correlation with propionate concentration by simple linear regression analysis. It was found that acetic acid and propionate concentrations were raised by the increase of the bacterial count of *Bacteroides* and *Fusobacterium*. Furthermore it was suggested that it was expected that the variability of these things repairs the enteral immune mechanism. Hence, it was found to have to give SCFA production a high bacterial strain such as *Bacteroides* and *Fusobacterium* of the specificity to raise the SCFA concentrations in the intestinal tract for symptomatic LPE with dogs.

Chapter 4. Clinical application of FMT in the symptomatic LPE dog.

In Chapter 2, it was found that a ratio of the Fusobacteria phylum significantly decreased and a ratio of Bacteroidetes phylum in a tendency to decrease in the symptomatic LPE dogs. Furthermore, in Chapter 3, it was found for acetic acid and propionate production that Fusobacterium and Bacteroides were high specificity bacterial species. Hence, we thought that it was able to give SCFA production these high bacterial strains of the specificity to raise the SCFA concentrations in the intestinal tract for symptomatic LPE with dogs by FMT. Therefore we conducted clinical application of FMT for the symptomatic LPE dogs to be resistant to treatment with drugs.

To evaluate clinical safety of FMT, 4 aLPE dogs underwent 4 times FMT procedure once every 3 weeks. The clinical safety was evaluated by activity, appetite, frequency of vomiting, feces property, frequency of the defecation, weight and the blood chemical examination about the liver. The abnormality was absent to a safe index after FMT procedure. Therefore, we could confirm if it was less than 4 times or 84 days, FMT by the enema was provided safely.

We performed FMT for 9 sLPE dogs and investigated the change of clinical manifestations to evaluate clinical efficacy of FMT. The changes in clinical signs (e.g., vomiting, diarrhea, and the feces property) were evaluated according to CIBDAI and Waltham Faeces Scoring system. The score of these index significantly decreased in all dogs (respectively, $p < 0.05$) with improvements in clinical signs. Furthermore, in the sLPE dogs after FMT procedure, treatments of an antimicrobial agent and the corticosteroid became needless.

Here, we performed FMT in 9 sLPE dogs using the fecal matter of donor dogs and investigated the subsequent changes in the fecal microbiome and clinical signs. In 2 of sLPE dogs and their 2 of donor dogs, the fecal microbiome was examined by 16S rRNA sequencing. In the sLPE dogs, α diversity of the microbiome significantly decreased compared it after donor dogs and after FMT ($p < 0.03$). In β diversity of the microbiome of the sLPE dogs after FMT resembled their donor dogs, while difference characteristics were found between these both groups and sLPE group before FMT procedure. Moreover, our observations confirmed that changes in the fecal microbiome diversity indeed affected the sLPE dogs, and hence, contributed to the efficacy of FMT treatment. The predominant bacterial phylum was Bacteroidetes (47.3% and 40.3%), Firmicutes (24.6% and 24.5%) and Fusobacteria (20.2% and 22.6%) of total bacterial population in the donor dogs. After the FMT procedure, there was a decrease in the proportion of Proteobacteria and an increase in the proportion of Fusobacteria and other dominant bacterial groups (Firmicutes and Bacteroidetes). It was suggested that FMT for the sLPE dogs show effect of treatment by restoring dysbiosis, because the fecal microbiome of sLPE dogs was similar to the donor’s fecal microbiome.

Enterobacteriaceae and *Fusobacterium* were detected in the 9 sLPE dogs by quantitative PCR. After FMT fecal microbiome, the bacterial counts of *Enterobacteriaceae* was significantly decreased, while the bacterial counts of *Fusobacterium* was significantly increased (respectively, $p < 0.01$). In these result, it was suggested that it was a characteristic bacteriological change in the dog of LPE. The microbiome of sLPE dogs is a state of dysbiosis from these, thus it is thought to be important to use phylum-rich (e.g., Bacteroidetes, Firmicutes and Fusobacteria) feces transplant liquid to improve this dysbiosis. Because all treatments, especially with antibiotics, were discontinued during FMT, we are confident that pharmacotherapy did not affect the fecal microbiome. Also, in FMT, the likelihood that was the regimen that there were fewer side effects than treatment with drug, and was effective was suggested as a treatment for the LPE dogs.

Based upon the foregoing, the sLPE dogs were a state of dysbiosis, and the possibility that this state is involved in the clinical status mainly on the digestive symptom is suggested. The microbiome of sLPE dogs is a state of dysbiosis from these, thus it is thought to be important to use phylum-rich (e.g., Bacteroidetes, Firmicutes and Fusobacteria) feces transplant liquid to improve this dysbiosis. Furthermore, conventional treatment, consisting of an anti-flatulent agent, an antidiarrheal agent, antibiotics, and anti-inflammatory drugs did not improve these sLPE dog’s symptoms of diarrhea and vomiting. The use of FMT was employed and the symptoms improved after FMT treatment. In conclusion, we show the safety and efficacy of FMT in the sLPE dogs. We conclude that FMT should be considered as a treatment option for canine symptomatic LPE cases in the future.