

# *Giardia Duodenalis* Colonization Slightly Affects Gut Microbiota And Haematological Parameters In Clinically Healthy Dogs

**Arianna Peruzzo**

Istituto Zooprofilattico Sperimentale delle Venezie

**Marta Vascellari**

Istituto Zooprofilattico Sperimentale delle Venezie

**Andrea Massaro**

Istituto Zooprofilattico Sperimentale delle Venezie

**Marzia Mancin**

Istituto Zooprofilattico Sperimentale delle Venezie

**Annalisa Stefani**

Istituto Zooprofilattico Sperimentale delle Venezie

**Massimiliano Orsini**

Istituto Zooprofilattico Sperimentale delle Venezie

**Patrizia Danesi**

Mycology and Medical Entomology

**Sara Petrin**

Istituto Zooprofilattico Sperimentale delle Venezie

**Antonio Carminato**

Istituto Zooprofilattico Sperimentale delle Venezie

**Michele Matteo Santoro**

Corso Allevamento e Addestramento Cinofili

**Roberto Speranza**

Corso Allevamento e Addestramento Cinofili

**Carmen Losasso** (✉ [closasso@izsvenezie.it](mailto:closasso@izsvenezie.it))

Istituto Zooprofilattico Sperimentale delle Venezie

**Gioia Capelli**

Mycology and Medical Entomology

---

## Research Article

**Keywords:** Dogs, *Giardia duodenalis*, Gut, Lipid metabolism, Microbiota.

**Posted Date:** February 11th, 2022

**DOI:** <https://doi.org/10.21203/rs.3.rs-1334354/v1>

**License:**  This work is licensed under a Creative Commons Attribution 4.0 International License.

[Read Full License](#)

---

# Abstract

*Giardia duodenalis* (*G. duodenalis*) is a worldwide cause of acute diarrheal disease both in humans and in animals. Domestic dogs may either harbor the parasite subclinically or showing the infection typical clinical signs. However, because giardiasis is a recognized veterinary threat, testing for this agent is often performed when canine patients are presented to veterinarians. Usually, animals are treated with antiparasitic agents in case of positive test, regardless of the severity of enteric clinical signs. Here we report for the first time a study comparing two groups of clinically healthy German shepherd dogs differing for *G. duodenalis* colonization. Gut microbiota, the haematological, biochemical and faecal parameters related to the intestinal function were investigated. The results display a scenario in which *G. duodenalis* exerts an effect upon the gut microbiota affecting the proportion of few bacterial taxa known to be associated with improved lipids metabolism and protection from gut inflammation. This also suggest that the antiparasitic treatments that are usually administered to *G. duodenalis* positive dogs might be avoided in clinically healthy subjects, since the presence of *G. duodenalis* does not substantially modify the microbial ecology of the intestinal lumen nor the haematological markers of disease.

## Introduction

*Giardia duodenalis* (*Giardia*) is a common worldwide parasite of both humans and domestic animals and it is currently recognised as the most prevalent gastrointestinal parasite in domestic dogs, closely followed by hookworms and coccidian [1–5]. The role of *Giardia* in causing a broad range of clinical manifestations, from asymptomatic to acute/chronic diarrheal disease, remains a matter of debate. Even though *Giardia* is frequently detected in diarrheic animals, particularly in puppies, many hosts remain asymptomatic despite shedding high numbers of environmentally-resistant cysts [6]. The main sustained hypothesis on giardiasis and associated sequelae is that the parasite attachment causes the loss of epithelial barrier function [7, 8], favouring the penetration of intestinal bacteria into the inflamed intestinal wall, resulting in a permanent damage to the intestinal epithelium [9]. Some authors postulated that changes in the resident intestinal microflora are responsible for the disease outcome with giardiasis [10]. However, the host-parasite interaction is not a one way process and changes in the host microbiome itself may favour the contact between parasites and host cells. Such changes may be caused by different stresses, such as nutritional or environmental changes, infections, or drug and antimicrobial treatments [6]. Thus, whether *Giardia* is a commensal or a parasite, it is conceivable that the perturbation of the host-parasite equilibrium may be the basis of some pathogenicity, and may explain variations in symptoms both between hosts and within the same host over time [6].

If host microbiota may be primarily or secondarily involved in *Giardia* infection outcomes, many factors other than *Giardia* may affect the gut microbiome, potentially masking the *Giardia*-microbiome relationship.

The primary aim of this study was to investigate the impact of *Giardia* infection on gut microbiota in a homogeneous population of dogs, living in the same breeding facility. Moreover, the haematological,

biochemical and faecal parameters related to the intestinal function were investigated.

## Results

### Sample description

Overall, 19 females and 12 males German Shepherd dogs were included. The median age was 19 months (range 15-85 months). Dogs ranged from 28 to 35 kg of body weight. All dogs were non-diarrhoeic and clinically healthy.

### *Giardia* detection and quantification

Overall, 13 dogs were positive for *Giardia* (GP), of which 8 were females (GPF) and 5 were males (GPM). All positive dogs ranged from 15 to 24 months (Table S1), showing a significant lower age distribution in *Giardia* positive dogs than negatives ( $p$ -value= 0.0003). Equivalent conclusions were inferred considering the age distribution within females ( $p_{\text{adj}}$ -value= 0.021) and males ( $p_{\text{adj}}$ -value= 0.046). The same percentage of *Giardia* infection was observed among males and females (42%). The number of *Giardia* cysts per 1g of faecal sample was higher than 50,000 in 6 dogs, between 10,000 and 50,000 in 4 dogs, and less than 10,000 in 3 dogs (Table S1). *Cryptosporidium* spp. was not detected in all the examined samples.

### Effect of *Giardia* infection on gut microbial community ecology

After data preprocessing, a total of 705,154 reads ( $n=31$ , mean for sample=22,746.9, SD=7254.902) were retained for bioinformatic analyses. The number of identified OTUs ranged between 200 and 600 if considering the entire sample with the exception of dog number 9 presenting a number of identified OTUs higher than 1000 (data not show).

No significant differences were found in the number of OTUs between GP and GN. Focusing on gender, no significant differences were found between GPF and GNF and between GPM and GNM. Considering the positive dogs, no significant differences were found in the number of OTUs between GPF and GPM while the number of OTUs was significantly higher in GNF than in GNM ( $p_{\text{adj}}$ -value= 0.011) (Figure 1).

Alpha diversity was analysed using Chao1 and Shannon indices. Chao1 indices were  $470.11 \pm 94.93$  (GP),  $596.93 \pm 501.66$  (GN),  $495.80 \pm 60.66$  (GPF),  $429.88 \pm 129.96$  (GPM),  $765.38 \pm 584.98$  (GNF) and  $332.22 \pm 94.70$  (GNM) while Shannon indices were  $7.90 \pm 0.73$  (GP),  $7.89 \pm 0.78$  (GN),  $8.07 \pm 0.45$  (GPF),  $7.65 \pm 1.06$  (GPM),  $8.32 \pm 0.5$  (GNF),  $7.20 \pm 0.64$  (GNM). No statistically significant difference between GP and GN groups with respect to both Chao1 and Shannon indices was observed. The same result was found when comparing GPF and GNF and between GPM and GNM. Focusing on positive dogs, no statistically significant difference was observed between GPF and GPM for both tested indices. Whereas, comparing GNF and GNM, the alpha diversity indices Chao1 and the Shannon are significant higher in GNF than GNM ( $p_{\text{adj}}$ -value= 0.0057 and  $p_{\text{adj}}$ -value= 0.043, respectively) (Figure 1).

The unique OTUs of different groups were summarized according to the result of OTU clustering analysis in Table 1. The OTUs table is reported in Table S2.

The beta diversity index between the GP and GN groups was significantly different ( $p$ -value=0.0025). The same results were found in the comparison between GNF and GPF ( $p_{\text{adj}}$ -value=0.02), whereas no statistically significant difference was observed between GNM and GPM. Focusing on negative dogs, the beta diversity index was different between the GNF and GNM groups ( $p_{\text{adj}}$ -value=0.02), while no statistically significant differences were found between GPF and GPM groups.

To display the proportion of different taxa at the class level, the bar plot reported in Figure 2 was generated based on the relative abundance of taxa. The results showed that 90% of microorganisms in the faecal samples of the entire dataset belonged to seven classes (Bacilli, Bacteroida, Betaproteobacteria, Clostridia, Erysipelotrichi, Fusobacteria, Gammaproteobacteria) (Figure 2A).

The comparison between GNF and GNM groups revealed the presence of 3 classes unevenly distributed: Gammaproteobacteria, Bacteroida and Fusobacteria. The Gammaproteobacteria were found with a higher frequency in the GNM group than the GNF one; on the contrary the two classes of Bacteroida and Fusobacteria displayed a higher frequency in GNF than in GNM (Figure 2B). Very similar distribution in the relative taxa frequency was found between GPF and GPM groups (Figure 2C).

The Figure 3 reports the results of volcano plots identifying the OTUs differing significantly ( $p < 0.05$ ) between GN and GP (a), GNF and GPF (b) and GNM and GPM (c). The outputs of the PLS-DA analysis, applied to the previous identified significant OTUs, were shown by using the scores plots and the variable importance in projection plots (Figure 4). Moreover, in supplementary results, the volcano plots and the outputs of the PLS-DA, applied to GPF and GPM (a) and GNF and GNM (b) were showed (Figure 1S and Figure 2S, respectively). All plots displayed a good discriminatory power, explaining from more than 50% (Figure 4 (a)) to about 80% (Figure 2S (b)) of the total variance of the models. The most relevant variables, corresponding to coefficient values greater than 80 for each of the five pairwise comparisons, were reported in Table 2. The analysis revealed significant differences in the relative abundance of specific taxa between positive and negative dogs with specific reference to Bacteroidales order that was found more abundant in *Giardia* positive dogs. In addition, when the positive and negative groups were broken down by gender, significant differences emerged. Specifically, within the female group, GP dogs displayed more abundance of Erysipelotrichales and Bacteroidales orders than GN ones. Within the male group, GN dogs were mainly colonized by Clostridiales than GP. Comparing the gender, GPM showed higher abundance of Clostridiales and Lactobacillales with respect to GPF and a higher abundance of Erysipelotrichales were found in both GPM and GPF, probably referring to different families or genus. As regards GNF, they were characterized by a higher abundance of Burkholderiales with respect to GNM, that displayed a higher abundance of Clostridiales compared to GNF (Table 2).

## Haematological and biochemical analysis

All the tested haematological and biochemical parameters were within the range of normal values in all dogs (Table S1). The volcano plot analysis showed no significant differences in haematological and biochemical parameters between GP and GN dogs (data not show). Focusing on gender subgroups, statistically significant higher levels of Triglycerides (TG) were observed among GNF if compared with GPF (Figure 6, p-value=0.0051), while, at the significance limit, GPM had higher levels of cCRP than GNM (Figure 6, p-value=0.0725). Considering the *Giardia* negative dogs, no differences were observed in the haematological and biochemical parameters between males and females. Instead, among the *Giardia* positive dogs, the GPF had significantly higher levels of lipase than GPM (p-value=0.0429) and significantly lower levels of cCRP (p-value=0.0204) (Figure 3S and Figure 4S).

## Discussion

The primary aim of this study was to investigate possible variations in gut microbiota in a population of asymptomatic dogs, naturally infected or not by *Giardia*; secondarily, we investigated possible variations in some haematological, biochemical and faecal parameters, in respect to *Giardia* infection.

Few previous studies aimed to investigate the relationship between *Giardia* and gut microbiota structure and composition in dogs [11–14]. However, the majority of these studies were affected by different possible confounders such as uncontrolled life styles (stray dogs), different origin or breeding of dogs, concurrent parasitic infections, different clinical signs, and anthelmintic treatments [11, 12, 14]. In order to minimize potential bias, this study was performed on a homogeneous population, belonging to the same breed, housed in the same conditions and fed with the same commercial maintenance dry food. Moreover all dogs included in the present study were clinically healthy, no therapies against *Giardia* or bacteria were administered in the previous two months and coprological investigations excluded the co-infection with other intestinal parasites.

In our survey, the overall percentage of *Giardia* infection was 42%, with no differences between male and female dogs. Prevalence rates of *Giardia* infection in dogs vary depending on the population under study and diagnostic method used, and can be as high as 45% [15]. Similar prevalence in shelter and commercial kennels were previously reported [4, 5, 16, 17]. We found that infected dogs were younger than negative ones, and this was an expected finding, since previous reports showed that younger dogs have a higher risk of *Giardia* infection than older ones [6, 17–21]. Similarly, it has been demonstrated that children are more likely to suffer from clinical infections than adults [22]. Differences in susceptibility to *Giardia* infection among different age groups is likely due to age-related shifts in microbiota composition as well as variability of hosts' immune factors [23].

Even though the precise mechanisms that undergo *Giardia* pathogenesis are incompletely understood, a pivotal role has been postulated for microbiota by recent research [24]. Functional and compositional modifications of gut microbiota have been demonstrated during the course of *Giardia* infection with particular reference to changes of microbial community biodiversity and altered species abundance [25]. In our investigation some significant differences in terms of microbial diversity were observed among

tested groups. Particularly *Giardia* infection was associated with a significant shift of beta-diversity substantiated by a relevant reduction of Gammaproteobacteria and an increase of Fusobacteria in GPM if compared with GNM. This highlights also a reduction of the differences in terms of microbial abundance between males and females in the presence of the infection. The reduction of Gammaproteobacteria and the increase of Fusobacteria have been observed in other experimental settings with regards to positive asymptomatic dogs [13].

Moreover the PLS-DA analysis displayed a significant imbalance of different bacterial taxa with particular reference to OTUs referred to the Erysipelotrichales, Lactobacillales, Clostridiales and Burkholderiales orders, with the first two being present with a higher extent in *Giardia* positive dogs.

Both Erysipelotrichales and Lactobacillales orders are of particular interest in this specific context. The occurrence of Erysipelotrichales in *Giardia* positive dogs could be strongly related with the progression of the infection. Indeed, many researches have addressed the importance of *Erysipelotrichaceae* in inflammation-related disorders of the gastrointestinal tract so far, with particular reference to human context. *Erysipelotrichaceae* abundance levels were found to be increased in the lumen of colorectal cancer patients as compared to healthy controls [26], and to be significantly higher in the tumour group of an animal model of 1, 2-dimethylhydrazine-induced colon cancer [27]. However a high inter-host variation has been observed so far [24] probably due to the inherent differences species related in the gut microbiota (i.e. mice and humans) and/or differences in the immune responses upon sensing bacterial ligands [28, 29]. Moreover many studies have demonstrated the association between this bacterial order and host lipid metabolisms [26, 27, 30–33].

As regards to the order of Lactobacillales, it is well known their involvement in contrasting infections-induced gut oxidative stresses [34] and their involvement in preventing the adherence of *Giardia* trophozoites to the mucosal surface [35]. These findings draw a possible scenario in which the microbiota of *Giardia* positive subjects on one hand metabolically supports the persistence of *Giardia*, by means of *Erysipelotrichaceae* abundance, and on the other hand, counteracts the host's inflammation caused by *Giardia* itself by means of Lactobacillales action.

In our study all the biochemical parameters were within the normal ranges, and a significant differences were observed between *Giardia* positive and negative dogs, with respect to gender. Particularly, among *Giardia* positive dogs, serum lipase was significantly higher in females than in males. Moreover, GPF showed significantly lower values of triglycerides than *Giardia* negative females. These findings may be linked to the *Giardia* limited ability to synthesize lipids for membranes and organelles biosynthesis, energy production and growth [36]. Thus, lipids have to be provided by the host itself and / or its gut microbiota through the conversion of primary bile acids to secondary bile acids [37]. As a consequence, the host lipid metabolism during giardiasis might have a key role in keeping the parasite persistent in the intestinal lumen [13].

When a host ingests the cysts, these enter the digestive tract where they are stimulated by the acidic milieu in the stomach and the presence of bile and trypsin in the duodenum to develop into motile

trophozoites in the proximal small intestine. In the upper intestinal tract, the trophozoites proliferate and use their adhesive disc to attach to the intestinal villi [38]. As the parasite density increases and the trophozoites descend into the lower intestinal tract, they encounter decreased cholesterol, increase in pH, and increased concentrations of bile and lactic acid [39]. These conditions promote trophozoites differentiation into infectious cysts that are released into the environment [39]. Moderately enhanced bile acid concentration has been found to promote *Giardia* growth *in vitro* [37]. The mechanism by which bile stimulates parasite growth is unknown, but uptake of conjugated bile salt by *Giardia* could reduce intraluminal bile salt concentrations and possibly interfere with micellar solubilisation of fat and *Giardia*-bile salt interactions *in vitro* and *in vivo* [40]. Moreover, *Giardia* infection is associated with malabsorption of fats, due to mechanical mucosal damage, leading to intestinal steatosis and increased transit of lipids into the distal small intestine and colon [41].

In our study we investigated different functional (cobalamin, folate) and biochemical (C-reactive protein, calprotectin, alkaline phosphatase) biomarkers, that have been shown to be useful indicators of intestinal inflammation in dogs [42].

Interestingly, *Giardia* positive males displayed significantly higher values of cCRP than negative males as well as than positive females. Even though values remained within the reference range, this finding supports the presence of a pro-inflammatory state in these subjects. Differently, no significant differences among groups were observed in the values of the other investigated parameters. Taken together these findings are coherent with the absence of clinical symptoms, and allow to exclude severe gastro-intestinal inflammation in the investigated dogs, reinforcing the possibility of a delicate *Giardia*-microbiome-host equilibrium that may prevent the clinical manifestations.

Investigating *Giardia* ecological milieu in relation to the host's resident microbial community and its metabolic context is a very challenging task as it requires to take into consideration homogeneous cohorts that do not show evident signs of disease, such as diarrheal syndrome and intestinal malabsorption, known to compromise the luminal microbial environment and the serum biochemical profile.

## Conclusion

Here we report for the first time a study comparing a group of *Giardia* negative dogs with a group of *Giardia* positive but clinically healthy subjects. The main strength of this study is the lack of confounders such as different breeding and nutrition of dogs, concurrent parasitic infections, and clinical signs. On the other hand, a possible limit is represented by the low number of dogs considered, that may have prevented to observe further differences in the study groups.

Our results showed that the presence of *Giardia* exerts an effect upon the gut microbial communities, enriched in protective taxa against gut inflammation and depleted of lipids producing taxa, potentially usable to limit *Giardia* infection and relieve host inflammation.

Taking together the outcomes of our study suggest that treatments against *Giardia* should be considered with caution in clinically healthy subjects, in order to save the *Giardia*-microbiome-host equilibrium.

## Material And Methods

### Sample description

Thirty-one German shepherd dogs, living in the same breeding and training facility of the Italian Finance Police, were included in the study. The dogs were housed in individual boxes and fed with the same commercial maintenance dry food. All dogs were annually vaccinated against the canine distemper virus (CDV), canine parvovirus (CPV), canine adenovirus (CAV) and Leptospirosis, regularly treated by anthelmintic drugs, and protected against ectoparasites by using a slow-release insecticidal and repellent collar. The inclusion criteria were: (i) clinically healthy, without evidence of gastro-intestinal disorders; (ii) no pharmacological therapy in the 2 months before the study; (iii) tested negative with a standard sedimentation-floatation coprological test. From each dog, the following samples were collected: a minimum of 10 g of faecal samples for parasitological and biochemical analysis; 2 independent biological replicates of rectal faecal swabs (FecalSwab™, Copan Diagnostics Inc, Brescia, Italy) for microbial community analysis; 1 K3-EDTA and 1 plain tubes of peripheral blood for haematological and biochemical investigations, respectively. Aseptic techniques and disposable equipment were used for each sample.

### *Giardia* and *Cryptosporidium* spp. detection and quantification

The detection of *Giardia* and *Cryptosporidium* spp. was performed in faecal samples using the commercially available immunofluorescence test according to manufacturer instructions (Merifluor® *Cryptosporidium*/*Giardia*, Meridian Bioscience). Quantification of *Giardia* cysts and *Cryptosporidium* spp. oocysts was made by counting protozoa elements under the microscope and expressed as a number of (oo-)cysts per 1g of faecal sample to a maximum of 50,000. Specimens with higher parasite (oo-) cysts were recorded in the report as > 50,000.

### Microbial community analyses

#### *DNA extraction*

Total DNA for metataxonomic analysis was extracted using a column-based kit (QIAamp DNA Mini Kit, QIAGEN, Hilden, Germany) starting from 200 µL of faecal sample in swab's buffer (Modified Cary Blair medium), following the manufacturer's instruction. Thermal lysis was carried out at 56°C for 2 h, and RNaseA (70 Kunitz units/mg protein) was added to each sample to ensure RNA-free preparation. Total DNA was resuspended in 200 µL of nuclease-free water and stored at -20 °C until library preparation for sequencing.

#### *16S rRNA sequencing*

Extracted DNA was used as a template in amplicon PCR to target the hypervariable V3 and V4 regions of the bacterial 16S rRNA gene. The amplification check was performed by 2% TAE agarose gel electrophoresis to identify a DNA fragment accounting for 550 bp length. The 16S library was prepared according to the Illumina 16S Metagenomic sequencing Library Preparation protocol, using the primers Bact341F and Bact785R (Fwd: CCTACGGGNGGCWGCAG and Rev: GACTACHVGGGTATCTAATCC) previously described by Klindworth A et al. [43] using the Nextera XT DNA Library Prep kit (Illumina). PCR clean-up was performed with Agencourt AMPure XP beads (Beckman Coulter Genomics, Indianapolis, IN, USA). Libraries were checked for both concentration and quality using Qubit and 2200 TapeStation (Agilent), respectively. Samples were equimolarity pooled, and sequencing was performed with an Illumina MiSeq platform using a MiSeq 600V3 cartridge (600 cycles, 2x300 bp, paired-end reads). Read sequences were deposited in the Sequence Read Archive (SRA) of the NCBI under the BioProject PRJNA736250.

### ***Reads Preprocessing and OTU Table Construction***

After sequencing, data underwent a quality control procedure using the FastQC tool [44]. Data were then cleaned by removing adapters, primers, and performing dereplication of sequences using an in-house bash script. In addition, data were filtered based on the quality and length of the reads, so that only reads with a quality higher than a given threshold (QPhred  $\geq$  20) and longer than 100 bp were retained. All subsequent steps were performed using QIIME2 pipeline version 2020.2 [45]. Raw sequence data was screened, trimmed, and denoised with DADA2 [46] (parameters: p-trunc-len-f=288; p-trunc-len-r=264) and quality filtered based on q-score. Operational taxonomic units (OTUs) were defined as sequences with at least 97% similarity with Greengenes database (last release May 2013, version 13.8) [47]. Samples were rarefied to 98197 sequences per sample. The rarefaction depth was based on the lowest read depth of samples.

### **Haematological and biochemical analysis**

Blood EDTA samples were used for complete blood cell count (CBC) with the haematology analyser XN-1000V (Sysmex Europe GmbH, Norderstedt, Germany), equipped with a veterinary software (Software of Automated hematology Analyzer for Animal XN-V series (Sysmex). All samples were analysed within 8 hours after blood collection. Blood plain tubes were centrifuged 3000xg for 10 minutes and serum samples were collected for biochemical analysis: routine biochemical profile analysis were performed using an automated clinical chemistry analyser (Cobas C501; Roche Diagnostics International AG, Rotkreuz, Switzerland); folate and cobalamin (B12) immunoassay analysis were performed with automated Cobas e601 analyser (Roche Diagnostics). Serum canine C reactive protein (cCRP) concentration was determined via a commercially available turbidimetric immunoassay kit (Turbovet canine CRP, Acuvet Biotech, Zaragoza, Spain) applied to Cobas c501 analyser, following manufacturer instruction.

Faecal calprotectin was determined via a species-specific ELISA kit (Canine Calprotectin CP, MyBioSource, San Diego, CA), following manufacturer instruction. Briefly, 10 mg of samples were homogenized in 100

$\mu\text{L}$  of PBS and centrifuged for 20 minutes at 1500xg; supernatant was carefully collected and stored at  $-80^{\circ}\text{C}$  until analysis. Faecal samples were then thawed and measured in a unique batch.

## **Statistical analyses**

The overall goal of the statistical analysis was to compare the gut microbiota and the investigated haematological and biochemical parameters, with respect the *Giardia* infection (*Giardia* Positive dogs (GP) and *Giardia* Negative dogs (GN)).

Supposing a possible effect of the dog gender, the above mentioned analysis were performed considering also the data stratified by gender of dogs. Therefore, the subgroups *Giardia* Positive females (GPF), *Giardia* Negative females (GNF), *Giardia* Positive males (GPM), *Giardia* Negative males (GNM) were also evaluate in the statistical analysis.

Due to limited number of dogs, an additional stratification for age was not carried out. Nevertheless, the information about dogs' age was used to check whether the investigated groups had a different age distribution by Wilcoxon test.

R 4.0.5 software [16], QIime2 [45] and MetaboAnalyst 5.0 web portal [49] were used to conduct the statistical analysis. P-value $<0.05$  was considered significant.

## ***Analysis of 16S rRNA gene***

Alpha diversity analysis was performed on the pre-processed count table and was measured by means of the Chao1 index to assess the richness, Shannon index to assess the evenness and observed OTUs metrics to describe the community structure. The non-parametric Wilcoxon test was used to compare the alpha diversity and the OTUs number distribution between the GP and GN dogs. In addition, the non-parametric Kruskal-Wallis test was conducted to compare the GPF, GNF, GPM and GNM groups. If significant, the pairwise comparison Wilcoxon test was performed adjusting the p-value ( $p_{\text{adj}}$ -value) for multiple comparisons using the false discovery rate (FDR) method [50].

Beta diversity was evaluated with the phylogeny based Unifrac distance metric. The PERMANOVA test (permutation number 999) was used to compare the beta diversity parameters among groups [51]. FDR correction for multiple testing was applied in pairwise comparisons between groups.

The volcano plot [52] was drawn to identify the OTUs differing significantly between the groups of study. The features resulting significant (p-value $<0.05$ ) were selected and analysed by partial least square discriminant analysis (PLS-DA) [53]. The most discriminating OTUs were shown in descending order of their coefficient scores. The ones with a coefficients score greater than 80 were identified and discussed.

## ***Haematological and biochemical analyses***

The volcano plot was built to identify the haematological and biochemical parameters that differing significantly between the groups of study. The significant variables ( $p < 0.05$ ) were selected and described by means of box plot.

## Declarations

### Ethics approval and consent to participate

This study was approved by the ethical committee of the Istituto Zooprofilattico Sperimentale delle Venezie (code CE-IZSVE 8/2019)

### Consent for publication

Not applicable

### Availability of data and materials

The datasets supporting the conclusions of this article are available in the NCBI repository, BioProject n. PRJNA736250 (<https://www.ncbi.nlm.nih.gov/bioproject/PRJNA736250>)

### Competing interests

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

### Funding

This work was funded by Italian Ministry of Health, Grant N. RC IZSVE 05/18

### Authors' Contributions

MV, CL, MM: Wrote the manuscript; GC: Provide funding; CL, GC, MV, AC: Conceived and designed the analyses; AP, MV, AS, PD, SP, AC: Collected the data; AP, AS, PD: performed the experiments; AP, AM, MM, MO: Performed the analyses; MV, GC, MMS and RS: Collected the sample; All authors discussed the results and contributed to the final manuscript.

### Acknowledgments

The authors wish to acknowledge Susanna Pasqualotto and Valentina Cagnin for their technical support

## References

1. Palmer CS, Traub RJ, Robertson ID, et al (2008) Determining the zoonotic significance of *Giardia* and *Cryptosporidium* in Australian dogs and cats. *Vet Parasitol* 154:142–147. <https://doi.org/10.1016/j.vetpar.2008.02.031>

2. Epe C, Rehker G, Schnieder T, et al (2010) Giardia in symptomatic dogs and cats in Europe-Results of a European study. *Vet Parasitol* 173:32–38. <https://doi.org/10.1016/j.vetpar.2010.06.015>
3. Halliez MCM, Buret AG (2013) Extra-intestinal and long term consequences of Giardia duodenalis infections. *World J. Gastroenterol.* 19:8974–8985
4. Capelli G, Frangipane di Regalbono A, Iorio R, et al (2006) Giardia species and other intestinal parasites in dogs in north-east and central Italy. *Vet Rec* 159:422–424. <https://doi.org/10.1136/vr.159.13.422>
5. Paoletti B, Iorio R, Capelli G, et al (2008) Epidemiological scenario of giardiasis in dogs from central Italy. In: *Annals of the New York Academy of Sciences*. Blackwell Publishing Inc., pp 371–374
6. Tysnes KR, Skancke E, Robertson LJ (2014) Subclinical Giardia in dogs: A veterinary conundrum relevant to human infection. *Trends Parasitol.* 30:520–527
7. Cotton JA, Beatty JK, Buret AG (2011) Host parasite interactions and pathophysiology in Giardia infections. *Int. J. Parasitol.* 41:925–933
8. Bartelt LA, Roche J, Kolling G, et al (2013) Persistent G. lamblia impairs growth in a murine malnutrition model. *J Clin Invest* 123:2672–2684. <https://doi.org/10.1172/JCI67294>
9. Chen TL, Chen S, Wu HW, et al (2013) Persistent gut barrier damage and commensal bacterial influx following eradication of Giardia infection in mice. *Gut Pathog* 5:. <https://doi.org/10.1186/1757-4749-5-26>
10. Singer SM, Nash TE (2000) The role of normal flora in Giardia lamblia infections in mice. *J Infect Dis* 181:1510–1512. <https://doi.org/10.1086/315409>
11. Šlapeta J, Dowd SE, Alanazi AD, et al (2015) Differences in the faecal microbiome of non-diarrhoeic clinically healthy dogs and cats associated with Giardia duodenalis infection: Impact of hookworms and coccidia. *Int J Parasitol* 45:585–594. <https://doi.org/10.1016/j.ijpara.2015.04.001>
12. Lee NN, Bidot WA, Ericsson AC, Franklin CL (2020) Effects of Giardia lamblia Colonization and Fenbendazole Treatment on Canine Fecal Microbiota. *J Am Assoc Lab Anim Sci* 59:. <https://doi.org/10.30802/aalas-jaalas-19-000113>
13. Berry ASF, Johnson K, Martins R, et al (2020) Natural Infection with Giardia Is Associated with Altered Community Structure of the Human and Canine Gut Microbiome . *mSphere* 5:. <https://doi.org/10.1128/msphere.00670-20>
14. Fujishiro MA, Lidbury JA, Pilla R, et al (2020) Evaluation of the effects of anthelmintic administration on the fecal microbiome of healthy dogs with and without subclinical Giardia spp. And Cryptosporidium canis infections. *PLoS One* 15:. <https://doi.org/10.1371/journal.pone.0228145>

15. Ballweber LR, Xiao L, Bowman DD, et al (2010) Giardiasis in dogs and cats: update on epidemiology and public health significance. *Trends Parasitol.* 26:180–189
16. Mundim MJS, Rosa LAG, Hortêncio SM, et al (2007) Prevalence of *Giardia duodenalis* and *Cryptosporidium* spp. in dogs from different living conditions in Uberlândia, Brazil. *Vet Parasitol* 144:356–359. <https://doi.org/10.1016/j.vetpar.2006.09.039>
17. Huber F, Bomfim TCB, Gomes RS (2005) Comparison between natural infection by *Cryptosporidium* sp., *Giardia* sp. in dogs in two living situations in the West Zone of the municipality of Rio de Janeiro. *Vet Parasitol* 130:69–72. <https://doi.org/10.1016/j.vetpar.2005.03.012>
18. Uiterwijk M, Nijssse R, Kooyman FNJ, et al (2019) Host factors associated with *Giardia duodenalis* infection in dogs across multiple diagnostic tests. *Parasites and Vectors* 12:. <https://doi.org/10.1186/s13071-019-3810-3>
19. Claerebout E, Casaert S, Dalemans AC, et al (2009) *Giardia* and other intestinal parasites in different dog populations in Northern Belgium. *Vet Parasitol* 161:41–46. <https://doi.org/10.1016/j.vetpar.2008.11.024>
20. Bouzid M, Halai K, Jeffreys D, Hunter PR (2015) The prevalence of *Giardia* infection in dogs and cats, a systematic review and meta-analysis of prevalence studies from stool samples. *Vet Parasitol* 207:181–202. <https://doi.org/10.1016/j.vetpar.2014.12.011>
21. Mircean V, Györke A, Cozma V (2012) Prevalence and risk factors of *Giardia duodenalis* in dogs from Romania. *Vet Parasitol* 184:325–329. <https://doi.org/10.1016/j.vetpar.2011.08.022>
22. Dhanabal J, Selvadoss PP, Muthuswamy K (2014) Comparative study of the prevalence of intestinal parasites in low socioeconomic areas from South Chennai, India. *J Parasitol Res* 2014:. <https://doi.org/10.1155/2014/630968>
23. Partida-Rodríguez O, Serrano-Vázquez A, Nieves-Ramírez ME, et al (2017) Human Intestinal Microbiota: Interaction Between Parasites and the Host Immune Response. *Arch. Med. Res.* 48:690–700
24. Fekete E, Allain T, Siddiq A, et al (2021) *Giardia* spp. and the Gut Microbiota: Dangerous Liaisons. *Front. Microbiol.* 11
25. Barash NR, Maloney JG, Singer SM, Dawson SC (2017) *Giardia* alters commensal microbial diversity throughout the murine gut. *Infect Immun* 85:. <https://doi.org/10.1128/IAI.00948-16>
26. Chen W, Liu F, Ling Z, et al (2012) Human intestinal lumen and mucosa-associated microbiota in patients with colorectal cancer. *PLoS One* 7:. <https://doi.org/10.1371/journal.pone.0039743>
27. Zhu Q, Jin Z, Wu W, et al (2014) Analysis of the intestinal lumen microbiota in an animal model of colorectal cancer. *PLoS One* 9:90849. <https://doi.org/10.1371/journal.pone.0090849>

28. Zschaler J, Schlorke D, Arnhold J (2014) Differences in innate immune response between man and mouse. *Crit Rev Immunol* 34:433–454. <https://doi.org/10.1615/critrevimmunol.2014011600>
29. Nguyen TLA, Vieira-Silva S, Liston A, Raes J (2015) How informative is the mouse for human gut microbiota research? *DMM Dis Model Mech* 8:1–16. <https://doi.org/10.1242/dmm.017400>
30. Erysipelotrichaceae - an overview | ScienceDirect Topics.  
<https://www.sciencedirect.com/topics/biochemistry-genetics-and-molecular-biology/erysipelotrichaceae>. Accessed 9 Jun 2021
31. Martínez I, Perdicaro DJ, Brown AW, et al (2013) Diet-induced alterations of host cholesterol metabolism are likely to affect the gut microbiota composition in hamsters. *Appl Environ Microbiol* 79:516–524. <https://doi.org/10.1128/AEM.03046-12>
32. Wu J, Liu M, Zhou M, et al (2021) Isolation and genomic characterization of five novel strains of Erysipelotrichaceae from commercial pigs. *BMC Microbiol* 21:1–12. <https://doi.org/10.1186/s12866-021-02193-3>
33. Kaakoush NO (2015) Insights into the role of Erysipelotrichaceae in the human host. *Front. Cell. Infect. Microbiol.* 5:84
34. Kong Y, Olejar KJ, On SLW, Chelikani V (2020) The potential of *Lactobacillus* spp. for modulating oxidative stress in the gastrointestinal tract. *Antioxidants* 9:1–15
35. Shukla G, Devi P, Sehgal R (2008) Effect of *Lactobacillus casei* as a probiotic on modulation of giardiasis. *Dig Dis Sci* 53:2671–2679. <https://doi.org/10.1007/s10620-007-0197-3>
36. Yichoy M, Duarte TT, De Chatterjee A, et al (2011) Lipid metabolism in *Giardia*: A post-genomic perspective. *Parasitology* 138:267–278
37. Riba A, Hassani K, Walker A, et al (2020) Disturbed gut microbiota and bile homeostasis in *Giardia*-infected mice contributes to metabolic dysregulation and growth impairment. *Sci Transl Med* 12:. <https://doi.org/10.1126/scitranslmed.aay7019>
38. FA L-R, J Y, RM B-C (2021) An update on cell division of *Giardia duodenalis* trophozoites. *Microbiol Res* 250:. <https://doi.org/10.1016/J.MICRES.2021.126807>
39. Lagunas-Rangel FA, Yee J, Bermúdez-Cruz RM (2021) An update on cell division of *Giardia duodenalis* trophozoites. *Microbiol. Res.* 250
40. Halliday CEW, Inge PMG, Farthing MJG (1995) Characterization of bile salt uptake by *Giardia lamblia*. *Int J Parasitol* 25:1089–1097. [https://doi.org/10.1016/0020-7519\(95\)00029-2](https://doi.org/10.1016/0020-7519(95)00029-2)

41. Farthing MJG (1993) Pathogenesis of giardiasis. *Trans R Soc Trop Med Hyg* 87:17–21. [https://doi.org/10.1016/0035-9203\(93\)90531-T](https://doi.org/10.1016/0035-9203(93)90531-T)
42. Heilmann RM, Steiner JM (2018) Clinical utility of currently available biomarkers in inflammatory enteropathies of dogs. *J. Vet. Intern. Med.* 32:1495–1508
43. Klindworth A, Pruesse E, Schweer T, et al (2013) Evaluation of general 16S ribosomal RNA gene PCR primers for classical and next-generation sequencing-based diversity studies. *Nucleic Acids Res* 41:. <https://doi.org/10.1093/nar/gks808>
44. Babraham Bioinformatics - FastQC A Quality Control tool for High Throughput Sequence Data. <https://www.bioinformatics.babraham.ac.uk/projects/fastqc/>. Accessed 9 Jun 2021
45. Bolyen E, Rideout JR, Dillon MR, et al (2019) Reproducible, interactive, scalable and extensible microbiome data science using QIIME 2. *Nat. Biotechnol.* 37:852–857
46. Callahan BJ, McMurdie PJ, Rosen MJ, et al (2016) DADA2: High-resolution sample inference from Illumina amplicon data. *Nat Methods* 13:581–583. <https://doi.org/10.1038/nmeth.3869>
47. DeSantis TZ, Hugenholtz P, Larsen N, et al (2006) Greengenes, a chimera-checked 16S rRNA gene database and workbench compatible with ARB. *Appl Environ Microbiol* 72:5069–5072. <https://doi.org/10.1128/AEM.03006-05>
48. Tonstad S, Stewart K, Oda K, et al (2013) Vegetarian diets and incidence of diabetes in the Adventist Health Study-2. *Nutr Metab Cardiovasc Dis* 23:292–299. <https://doi.org/10.1016/j.numecd.2011.07.004>
49. Pang Z, Chong J, Zhou G, et al (2021) MetaboAnalyst 5.0: narrowing the gap between raw spectra and functional insights. *Nucleic Acids Res.* <https://doi.org/10.1093/nar/gkab382>
50. Benjamini Y, Hochberg Y (1995) Controlling the false discovery rate: a practical and powerful approach to multiple testing. *J. R. Stat. Soc. B* 57:289–300
51. Estaki M, Jiang L, Bokulich NA, et al (2020) QIIME 2 Enables Comprehensive End-to-End Analysis of Diverse Microbiome Data and Comparative Studies with Publicly Available Data. *Curr Protoc Bioinforma* 70:e100. <https://doi.org/10.1002/CPBI.100>
52. Li W (2012) Volcano plots in analyzing differential expressions with mRNA microarrays. *J Bioinform Comput Biol* 10:. <https://doi.org/10.1142/S0219720012310038>
53. Brereton RG, Lloyd GR (2014) Partial least squares discriminant analysis: Taking the magic away. *J Chemom* 28:213–225. <https://doi.org/10.1002/CEM.2609>

## Tables

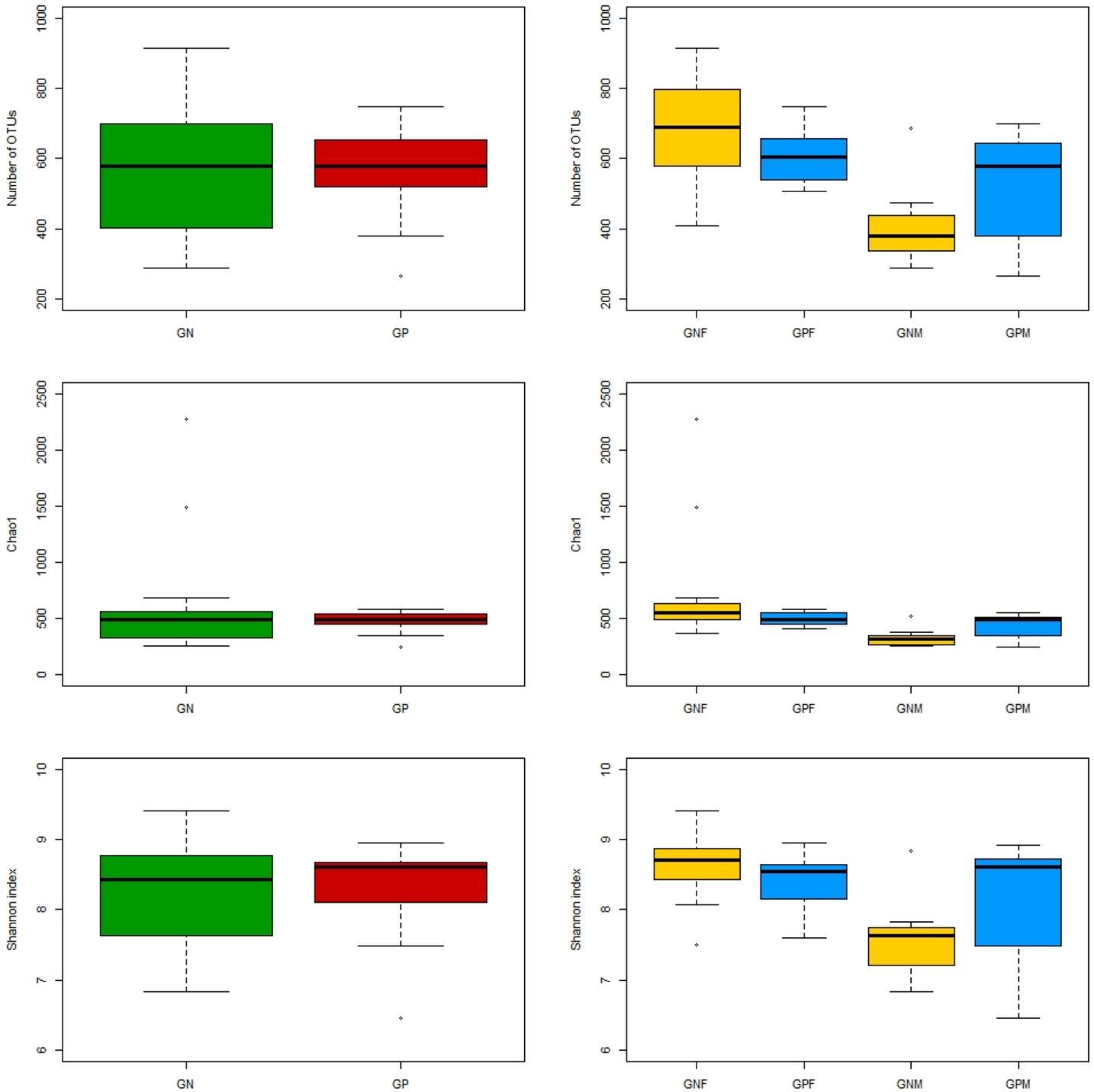
**Table 1:** unique OTUs per group

Groups	GP	GN	GPF	GPM	GNF	GNM
N.OTUs	2112	4863	754	652	3462	640

**Table 2:** differential abundance of microbial taxa among groups. In the “Group” column are reported the groups with higher abundance. GP= Giardia positive; GN=Giardia Negative; GPF= Giardia Positive Females; GPM= Giardia Postive Males; GNF= Giardia Negative Females; GNM= Giardia Negative Males

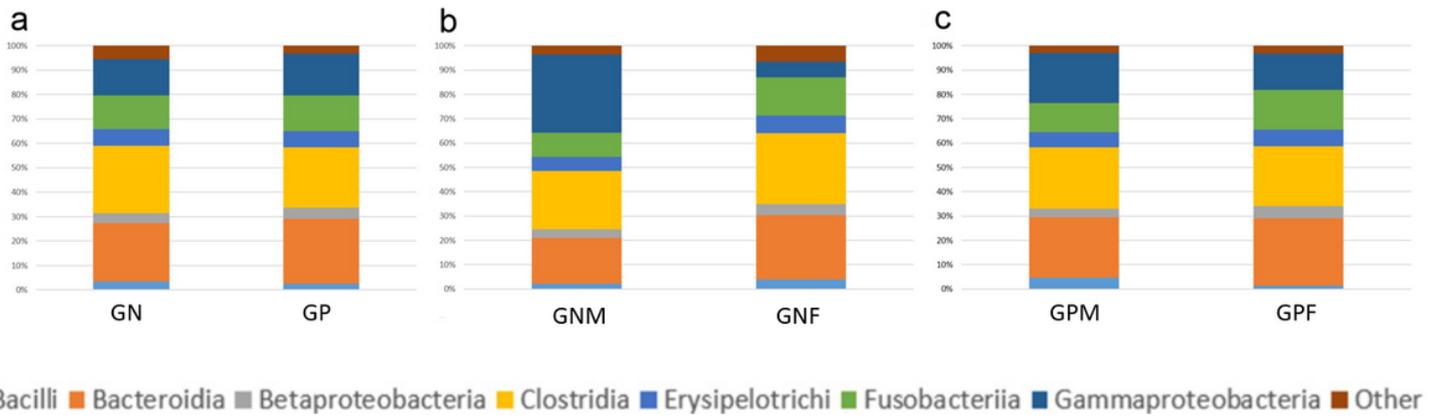
ID	Phylum	Class	Order	Group
<b>GN Vs GP</b>				
158	Bacteroidetes	Bacteroidia	Bacteroidales	GP
443	Bacteroidetes	Bacteroidia	Bacteroidales	GP
<b>GNF Vs GPF</b>				
147	Firmicutes	Erysipelotrichi	Erysipelotrichales	GPF
463	Bacteroidetes	Bacteroidia	Bacteroidales	GPF
<b>GNM Vs GPM</b>				
56	Firmicutes	Clostridia	Clostridiales	GNM
<b>GNF Vs GNM</b>				
45	Proteobacteria	Betaproteobacteria	Burkholderiales	GNF
52	Proteobacteria	Betaproteobacteria	Burkholderiales	GNF
56	Firmicutes	Clostridia	Clostridiales	GNM
<b>GPF Vs GPM</b>				
885	Firmicutes	Clostridia	Clostridiales	GPM
124	Firmicutes	Erysipelotrichi	Erysipelotrichales	GPM
201	Firmicutes	Clostridia	Clostridiales	GPM
238	Firmicutes	Clostridia	Clostridiales	GPM
105	Firmicutes	Bacilli	Lactobacillales	GPM
613	Firmicutes	Erysipelotrichi	Erysipelotrichales	GPF

## Figures



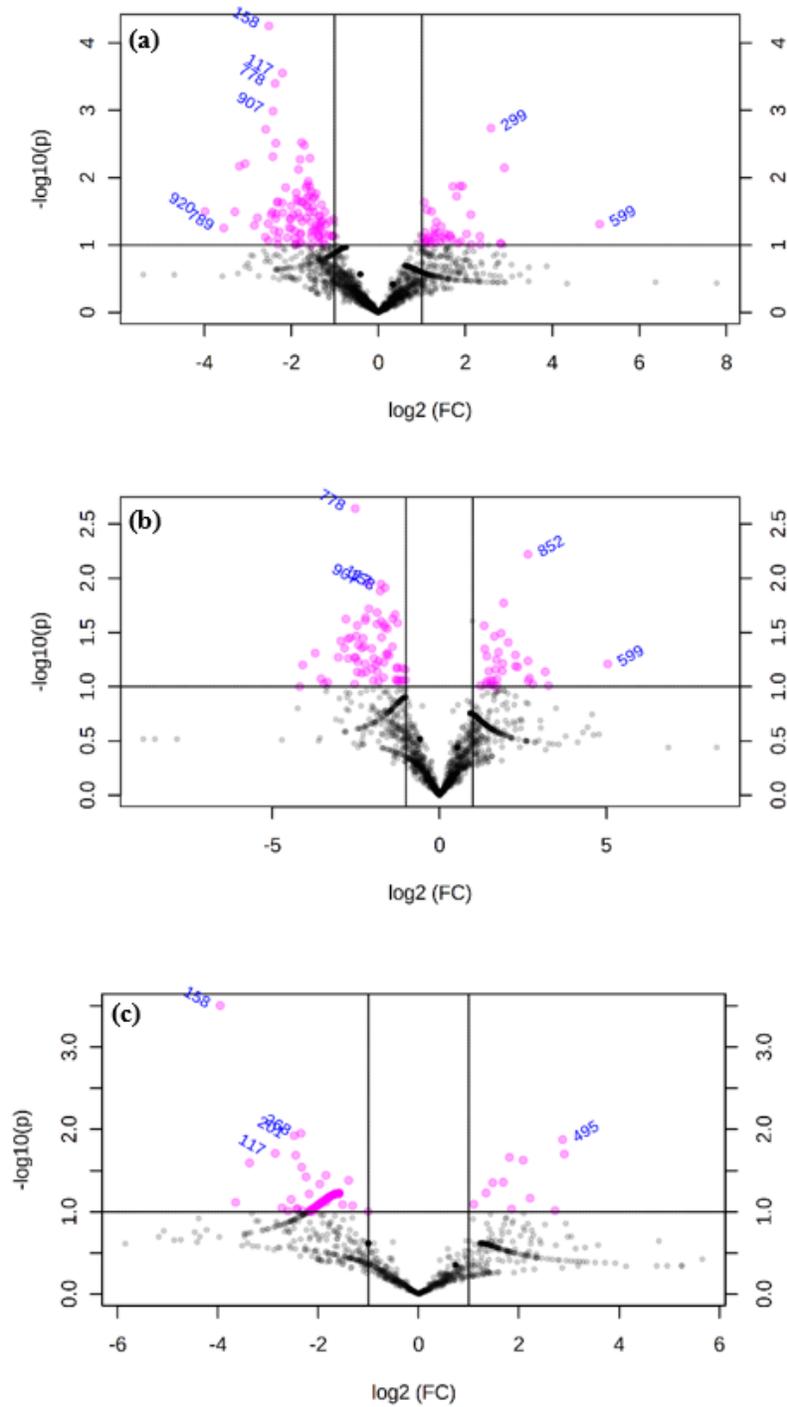
**Figure 1**

Box plot of the number of identified OTUs and Alpha-diversity (Chao1 and Shannon indices) by groups of study: GP and GN; GPF, GPM, GNF and GNM. The box plot synthesizes the data, providing the principal measures of central tendency and dispersion. Specifically, the diagram comprises a box with horizontal limits defining the upper and lower quartiles representing the interquartile range, with the median marked by a horizontal line within the box. The whiskers are vertical lines extending from the box as low as the 2.5<sup>th</sup> percentile and as high as the 97.5<sup>th</sup> percentile. Extreme values are indicated by dots.



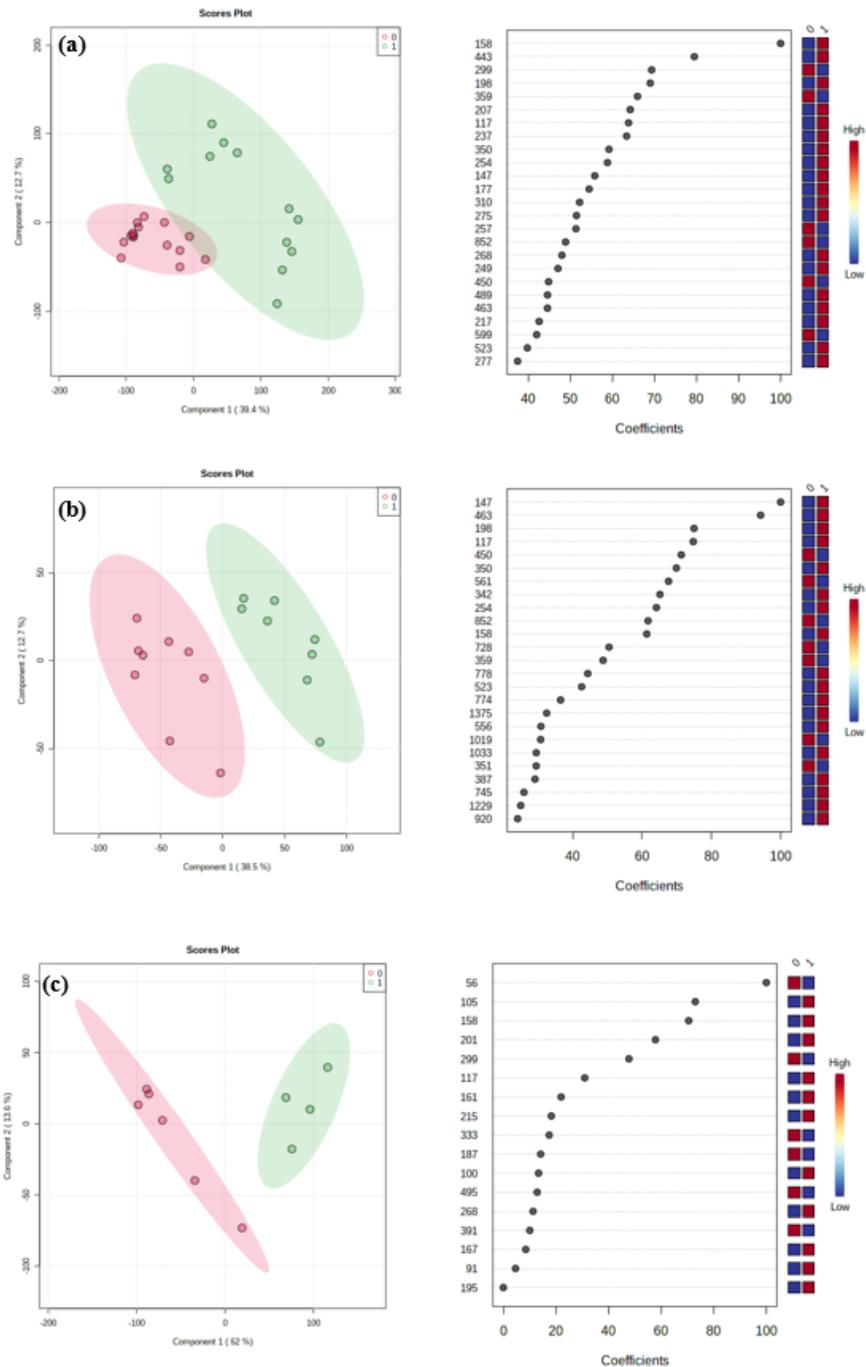
**Figure 2**

Barplots showing the average abundance of bacterial taxa at the order level between (a) GP and GN dogs, (b) GNM and GPF, (c) GPM and GPF.



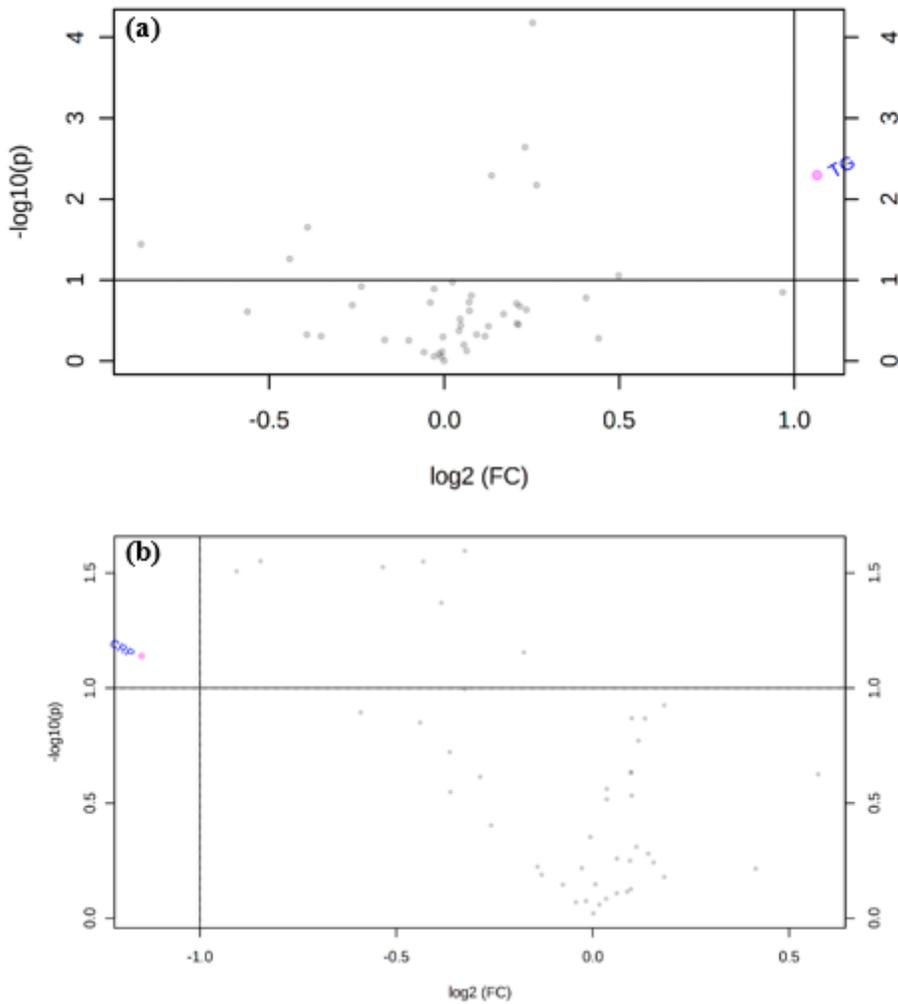
**Figure 3**

Volcano plot showing the OTUs that differ significantly between groups of study: (a) GP and GN, (b) GPF and GNF and (c) GPM and GNM. The pink points indicate variables-of-interest that display both large-magnitude fold-changes (x-axis) as well as high statistical significance ( $-\log_{10}$  of p-value Wilcoxon test, y-axis). The horizontal line shows the p-value cutoff (p-value = 0.10) with points above the line having p-value < 0.10 and points below the line having p-value > 0.1. The vertical lines shows 2-fold changes.



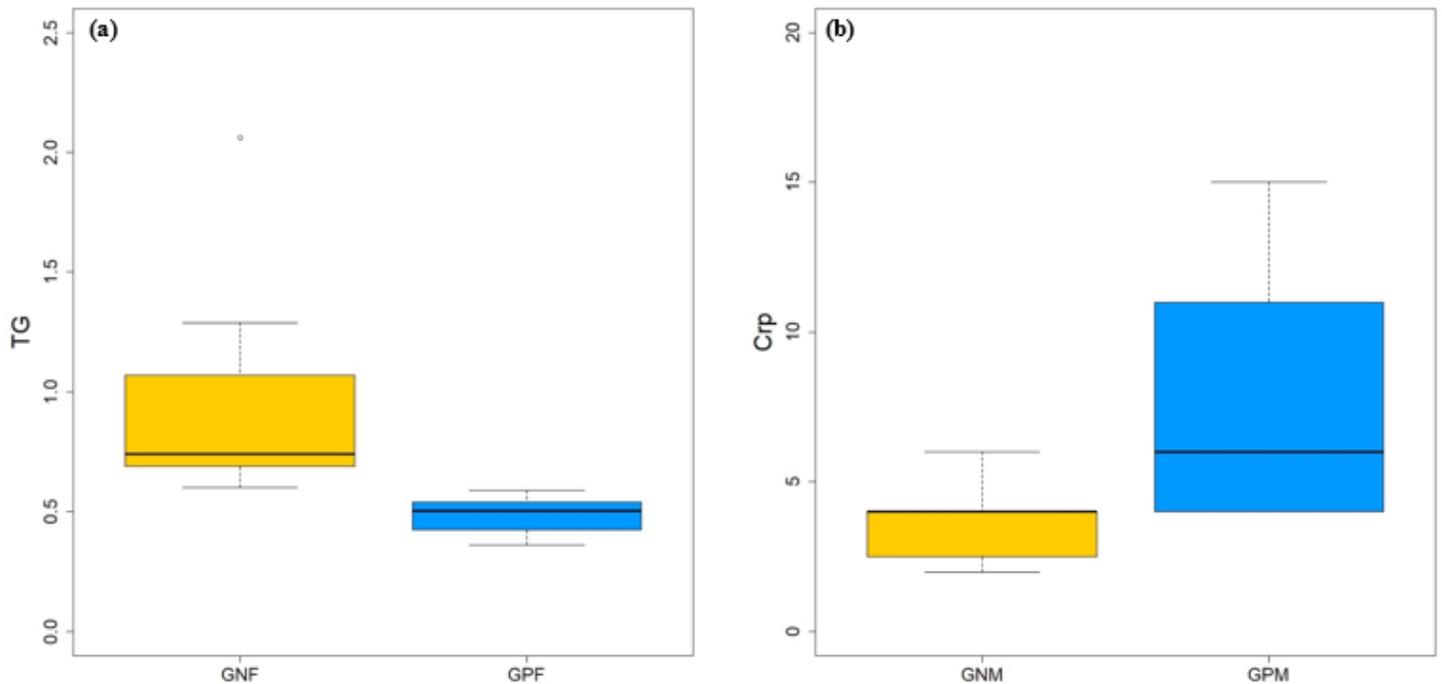
**Figure 4**

Partial least squares discriminant analysis (PLS-DA) results showing the comparison between OTU data acquired for (a) GP vs GN, (b) GPF vs. GNF and (c) GPM vs. GNM. On the left, the 2-D PLS-DA scores plots; on the right, the variable importance in projection plots. The most discriminating OTUs are shown in descending order of their coefficient scores. The colour boxes indicate whether OTU is increased (red) or decreased (blue) in positive (1) vs. negative (0)



**Figure 5**

Volcano plot showing the haematological and biochemical variables that differ significantly between groups of study: (a) GPF and GNF and (b) GPM and GNM. The pink points indicate variables-of-interest that display both large-magnitude fold-changes (x-axis) as well as high statistical significance ( $-\log_{10}$  of p-value Wilcoxon test, y-axis). The horizontal line shows the p-value cut-off (p-value = 0.10) with points above the line having p-value < 0.10 and points below the line having p-value > 0.1. The vertical lines shows 2-fold changes.



**Figure 6**

Box plot of the significant haematological and biochemical parameters in (a) female and (b) male dogs.

## Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- [Peruzzoetal.2021animalmicrobiomeTableSupplementary1.xlsx](#)
- [Peruzzoetal.2021animalmicrobiomeTableSupplementary2.csv](#)
- [Peruzzoetal.2021animalmicrobiomeSupplementaryFIGURE1.docx](#)
- [Peruzzoetal.2021animalmicrobiomeSupplementaryFIGURE2.docx](#)
- [Peruzzoetal.2021animalmicrobiomeSupplementaryFIGURE3.docx](#)
- [Peruzzoetal.2021animalmicrobiomeSupplementaryFIGURE4.docx](#)