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Effects of meglumine antimoniate and allopurinol treatment on the fecal microbiome profile in dogs with leishmaniosis

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Abstract

Background The combination of meglumine antimoniate and allopurinol is considered one of the most effective treatments for canine leishmaniosis caused by *Leishmania infantum*. This study investigated the effects of this treatment on the gut microbiome of 10 dogs from Spain, Portugal, and Italy via fecal shotgun metagenomic sequencing over six months.

Methods Dogs were sampled at baseline (BL), after one month of combined treatment with meglumine antimoniate and allopurinol (M1) and after six months of allopurinol treatment (M6). Fecal samples had their total DNA extracted and sequenced by Illumina sequencing. Posteriorly, a microbiome analysis was conducted to analyze bacterial abundance, diversity and enrichment.

Results The gut microbiome of *Leishmania*-infected dogs (BL) is dominated by *Prevotella*, *Collinsella*, *Bacteroides*, and *Blautia*, with individual variability being the primary determinant of microbiome composition. No significant changes in alpha diversity (Shannon index, gene number) or beta diversity (Bray-Curtis dissimilarity, UniFrac distance) were detected between pre- and post-treatment time points, suggesting that treatment with meglumine antimoniate and allopurinol does not disrupt the gut microbiota. Minor trends in taxonomic shifts were noted, with slight increases in *Bifidobacterium pseudocatenulatum*, *Collinsella tanakaei*, and *Slackia piriformis* after treatment, but these changes were not statistically significant after correction for multiple testing. Linear discriminant analysis and multivariable modeling confirmed that the microbial community structure was resilient to treatment effects. Individual-specific microbiome differences in diversity accounted for 52% of the observed variability, underscoring the personalized nature of the gut microbiota in dogs. Importantly, no adverse microbiome disruptions were detected, even with prolonged allopurinol use.

Conclusions This study highlights the robustness of the canine gut microbiome during antileishmanial therapy and highlights the use of meglumine antimoniate and allopurinol without compromising gut microbial diversity or health. Further studies with larger cohorts are recommended to confirm these findings and explore the functional roles of the gut microbiota in modulating immune responses in *Leishmania*-infected dogs.

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Keywords Dogs, Gut microbiota, *Leishmania*, Antimonial, Dysbiosis

Background

Canine leishmaniosis is caused by *Leishmania infantum*, a parasitic protozoan transmitted by the bite of phlebotomine sandflies [1]. The combination of N-methylglucamine (meglumine antimoniate) and allopurinol is considered the first-line and most efficacious treatment for canine leishmaniosis [2, 3]. The most frequent side effects described with the use of meglumine antimoniate in dogs are pain and swelling at the injection site, fever, diarrhea, loss of appetite and azotemia [3–8]. Allopurinol has also been associated with several associated effects, mainly urinary, including xanthinuria, kidney mineralization and urolithiasis [9, 10]. Moreover, owing to the overuse of such compounds, genetic bases for antileishmanial drug resistance have been positively selected [11], potentially decreasing drug efficacy.

Gut microbiome alteration can be related to diseases or drug side effects, which can change the functional composition and metabolic activities of bacteria in the gut and hence overall health status [12]. Gut microbiome alterations linked to *Leishmania* infection have been previously studied in a wide array of hosts with varying evidence. In mice, microbiome changes linked to *Leishmania* infection vary by breed, with the CsS/Dem breeds showing resistance and the OcB/Dem breeds being more sensitive [13]. However, in Syrian hamsters, no significant microbiome alterations are observed [14, 15]. In dogs, *Leishmania* infection increases *Proteobacteria* compared to healthy counterparts [16], while in humans, changes in *Ruminococcaceae* and *Gastranaerophilales* occur with visceral leishmaniosis [17]. Non-antibiotic drugs may also affect microbiomes [18], yet studies on antileishmanial treatments are limited. Miltefosine showed no effect in Syrian hamsters' microbiome [19], however, the effects of other antileishmanial treatments on the fecal microbiome remain unstudied.

Molecular tools are now the standard for microbiome analysis in dogs using feces, as it is the most accessible sample type in clinical settings [20, 21]. DNA shotgun metagenomic technique aims to sequence extracted DNA in a sample without prior amplification by PCR based on genomic DNA fragmentation and then randomly sequenced on a high-throughput sequencer. Unfortunately, this approach has rarely been used to assess the fecal microbiota in veterinary medicine [22, 23].

This clinical study aimed to identify whether the most recommended antileishmanial treatment in dogs does have an impact on fecal microbiome composition via shotgun sequencing approach.

Methods

Ethics

Dogs with confirmed leishmaniosis and not yet under treatment were eligible for the study. No untreated sick dogs were included in the study as a control group as it was against veterinarian ethical practice. Dog owners' written consent was obtained for fecal sampling and access to veterinarian records, if required. A basic questionnaire was given for dog lifestyle data.

Study design

The study was international, multicentric, prospective, controlled, randomized, and blinded. The inclusion criteria for each dog consisted of an appropriate diagnosis of leishmaniosis based on clinical signs and clinicopathological findings compatible with leishmaniosis and confirmation by positive quantitative serology, such as the indirect fluorescent antibody technique (IFAT) or enzyme-linked immunosorbent assay (ELISA), and/or identification of *Leishmania* parasites by cytology, histopathology, or polymerase chain reaction (PCR). Furthermore, dogs were not under treatment for leishmaniosis at the time of their first visit.

Several parameters were recovered for each enrolled dog: country of origin (Spain, Portugal, or Italy), date of sampling, sex, neutered status, breed, age, weight (kg), lifestyle (indoor, outdoor, or mixed), living conditions (city or countryside), cohabiting status, diet (commercial, homemade, or mixed) (Table 1).

Leishmaniosis treatment regime was homogeneous during the study and was based on the combination of subcutaneous meglumine antimonate (Glucantime®, Boehringer Ingelheim) at a dose of 50 mg/kg/12 h for 30 days, and oral allopurinol at 10 mg/kg/12 h for at least 6 months [3].

Among all three centers, 10 dogs were recruited that fulfilled the enrollment requirements. At each of the control points at diagnosis (BL) and after one and six months of treatment (M1 and M6), fecal samples were collected along with extra data on leishmaniosis state, LeishVet clinical stage [24], health comorbidities and other drug used unrelated to leishmaniosis (Table 2).

Microbiome DNA extraction from fecal samples

Fecal samples (stool swabs) were collected in Zymo-BIOMICS DNA/RNA Shield Collection Tubes with swab (R1106, Zymo Research Corporation; Los Angeles, CA, USA) to conserve the sample until shipment to laboratory facilities. Total DNA was extracted with the Zymo-BIOMICS DNA Miniprep Kit (D4300; Zymo Research

Table 1 Dog data that were included in the study. This table includes all dog-related data acquired for the study: sex, breed, age, neutered status, country of origin, lifestyle, habitat, possible cohabitation, and type of diet

Dog	Sex	Breed	Age (years)	Neutered	Origin	Lifestyle	Habitat	Cohabitation	Diet
Dog 1	Male	Boxer	1	No	Spain	Indoors	City	No	Commercial
Dog 2	Male	Dogue of Bordeaux	8	Yes	Portugal	Mixed	Countryside	No	Commercial
Dog 3	Male	Crossbreed	9	Yes	Italy	Mixed	City	No	Mixed
Dog 4	Female	Pointer	11	Yes	Spain	Mixed	Mixed	2 cats	Commercial
Dog 5	Male	Crossbreed	5	No	Italy	Mixed	Village	No	Mixed
Dog 6	Male	Crossbreed	1	Yes	Spain	Indoors	City	No	Commercial
Dog 7	Female	Crossbreed	11	Yes	Spain	Outdoors	Countryside	2 dogs	Commercial
Dog 8	Male	Golden Retriever	11	No	Portugal	Mixed	City	No	Commercial
Dog 9	Male	Crossbreed	11	Yes	Portugal	Mixed	Village	No	Commercial
Dog 10	Female	American Bully	6	Yes	Spain	Indoors	City	No	Commercial

Table 2 Medical data per dog at the time of sampling. Each dog was sampled three times, and their leishmaniosis was graded based on LeishVet stage [24], their comorbidities and non-leishmaniosis treatments were recorded

Dog	Sampling	LeishVet Stage	Comorbidities	Other treatments
Dog 1	BL	IV	Meningitis	Prednisone
	M1	II	Meningitis	Prednisone
	M6	IV	Meningitis	Prednisone
Dog 2	BL	II		Fenbendazole
	M1	II		
	M6	II		Telmisartan
Dog 3	BL	II		
	M1	II		
	M6	II		
Dog 4	BL	III	Ehrlichiosis	Doxycycline
	M1	I		
	M6	I		
Dog 5	BL	II		
	M1	II		
	M6	II		
Dog 6	BL	III		
	M1	II		Enalapril
	M6	O-I		Enalapril
Dog 7	BL	II		
	M1	I		
	M6	O		
Dog 8	BL	II	Hypothyroidism	Telmisartan and levothyroxine
	M1	II	Hypothyroidism	Telmisartan and levothyroxine
	M6	II	Hypothyroidism	Telmisartan and levothyroxine
Dog 9	BL	II	Skin lesions	Fluralaner and prednisolone
	M1	II		Fluralaner
	M6	II		
Dog 10	BL	III		
	M1	II-III		
	M6	O		

Corporation; Los Angeles, CA, USA) following the manufacturer's recommendations.

DNA sequencing

The extracted DNA was submitted to MacroGen (MacroGen, South Korea) for whole DNA shotgun metagenomic sequencing via an Illumina HiSeq 150 × 2 bp instrument,

obtaining a median depth of 10 million reads 150 bp paired end. Sequencing data was obtained in fastq format. The study sequencing data is available under NCBI BioProject PRJNA1199800 under BioSample records SAMN45888860, SAMN45892024 - SAMN45892052 and demultiplexed reads under SRR31760315- SRR31760344.

Sequence data analysis

Sequencing reads (fastq) were quality filtered and trimmed for indices and low-quality bases using Trimmomatic v0.39 (LEADING:30, TRAILING:30, MINLEN:75, SLIDINGWINDOW:30:20) [25]. Taxonomic classification of sequencing reads to taxa are analyzed by mapping them to marker genes with Metaphlan3 v201901b using the database HUMANN3 [26]. Gene Richness/Number was determined from alignment of high-quality reads using Bowtie2 v2.4.1 [27] against the Integrated Gene Catalog [28].

Taxonomy data were further analyzed with R v4.1.1 [29] and the packages phyloseq v1.38.0 [30], vegan v2.6.4 [31], lefser v1.4.0 [32], maaslin v1.8.0 (Mallick et al., 2021), and r_statix v0.7.0 [33]. Count data can be accessed by species, genera, and phyla in Supplementary information Table S1, Table S2 and Table S3.

Results

During 2021, 10 client-owned dogs sick of leishmaniosis met the inclusion criteria and were enrolled in the study at one of the three participating university veterinary teaching hospitals, four from Universitat Autònoma de Barcelona (Spain), four from Faculdade de Medicina Veterinária de Lisboa (Portugal) and two from Università di Napoli (Italy).

Seven were male (three entire and four neutered), and three were spayed female. The median age of the dogs enrolled was eight years (range one to 11 years). There were six breeds represented, including crossbreed ($n=5$) and 1 each of the following breeds: American Bully, Boxer, Golden Retriever, Pointer and Dogue of Bordeaux.

Microbiome composition of Leishmania-infected dogs

Taxonomic affiliation revealed a grand total of 198 different species divided into 82 different genera and 10 phyla for the 10 individuals during the six months of the study (Supplementary information: Additional file 1). An overview of the most abundant genera and species is displayed per dog ($n=10$) and time point (BL, M1 and M6) in Fig. 1.

Bacteria from the *Prevotellaceae* genus (mostly *Prevotella copri*) and the *Colinsella* genus (mostly *Colinsella intestinalis*) are dominant members of the microbiome in the *Leishmania*-infected dogs of this study, together with *Bacterioides* (mainly *Bacterioides vulgatus* and *Bacterioides intestinalis*) and *Blautia* genera (Fig. 1). The *Clostridium* genus is also present in many samples. Interestingly, *Colinsella intestinalis* shows lower abundance but higher prevalence than *Prevotella*. Similarly, *Ruminococcus gnavus* and *Bacterioides intestinalis* were frequently found in many of the samples (Fig. 1). Notably, *Escherichia coli* was present in 33% of the samples, with no apparent association with any of the considered variables, as well

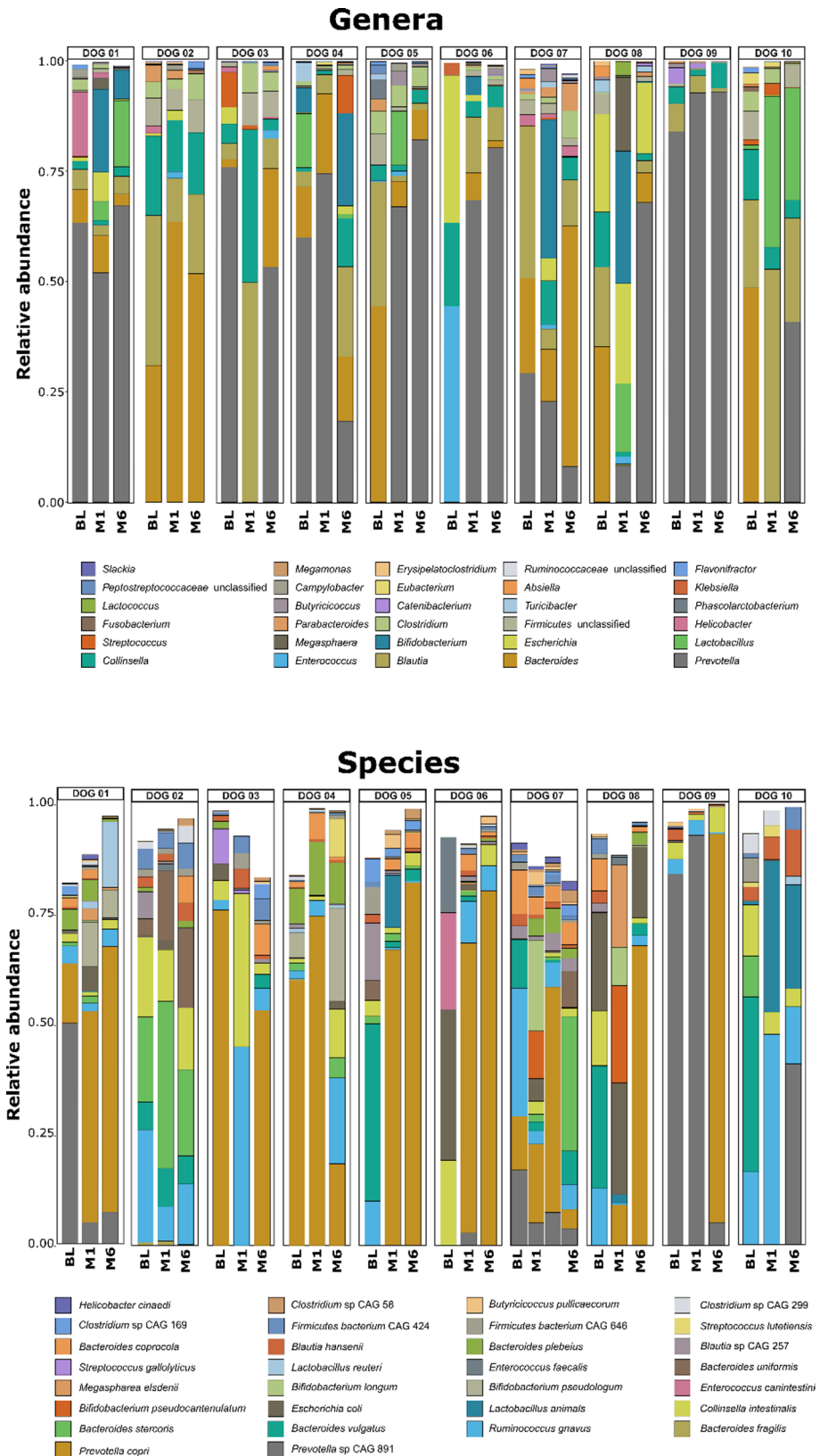
as the *Enterococcus* genus, which is highly abundant in Dog06.

Among the most frequent bacteria genera (top 15 at BL), *Blautia* and *Bacterioides* genera are the dominant members of the canine microbiome in *Leishmania*-infected dogs, followed by *Prevotella* and *Colinsella* genera (Fig. 2a). Likewise, the *Clostridium* genus was present all samples, and dominant in sample Dog06 in M1 and M6 (Fig. 1). Several bacterial genera identified as members of *Firmicutes* phylum were also worthy of note. *Escherichia* genus was present in 33% of the samples, with no apparent association with any of the considered variables (Fig. 2a). *Butyrucicoccus*, *Flavonifractor*, *Abisella*, *Catenibacterium* and *Streptococcus* genera, together with several unclassified genera of *Peptostreptococcaceae* family correspond to minor genera in the gut microbiome. Nevertheless, their relative abundance per sample strongly varies between baseline samples, highlighting the individual differences in dog gut microbiome. Among these most prevalent genera, the most representative bacterial species (top 15 at BL) corresponded to *Ruminococcus gnavus* and *Colinsella intestinalis*, followed by *Blautia henssenii* and several uncultured *Firmicutes* bacteria (Fig. 2b). *Escherichia coli*, *Bacterioides coprocola*, *Butyrucicoccus pullicaecorum*, *Bacterioides sterconis*, *Clostridium hiraonis*, *Flavonifractor plautii*, *Abisella dolichum* and *Bacterioides plebeius* are less represented species that are found in high abundance in some samples, such as *Escherichia coli*, which may correspond to dog specific microbiome composition.

A comparison of the microbiome communities of each sample and dog by weighted UniFrac distance showed no clear grouping by non-metric multidimensional scaling (Fig. 3). Bray-Curtis dissimilarities were statistically significant between individual dogs (PERMANOVA p -value=0.001) but not between sampled timepoints (PERMANOVA p -value=0.086), locations or any of the variables used in the study (Tables 1 and 2). The overall microbe structure depends more on the individual dogs (52% of the diversity) than on the timepoint. No overall differences in microbiome composition were detected for the duration of the study, as shown in Fig. 1.

Microbiome alteration due to antileishmanial treatment

To determine whether the combined administration of meglumine antimoniate and allopurinol during the first month of treatment impacts the gut microbiome, samples were analyzed as pairs (BL vs. M1). As shown in Fig. 4a, there were no significant differences in taxonomy (Wilcoxon p -value=0.36) or gene (Wilcoxon p -value=0.92) diversity (alpha diversity) before treatment and after a month of combined therapy. A comparison between the microbiome communities before and after one month of treatment (BL vs. M1) revealed no statistical difference due



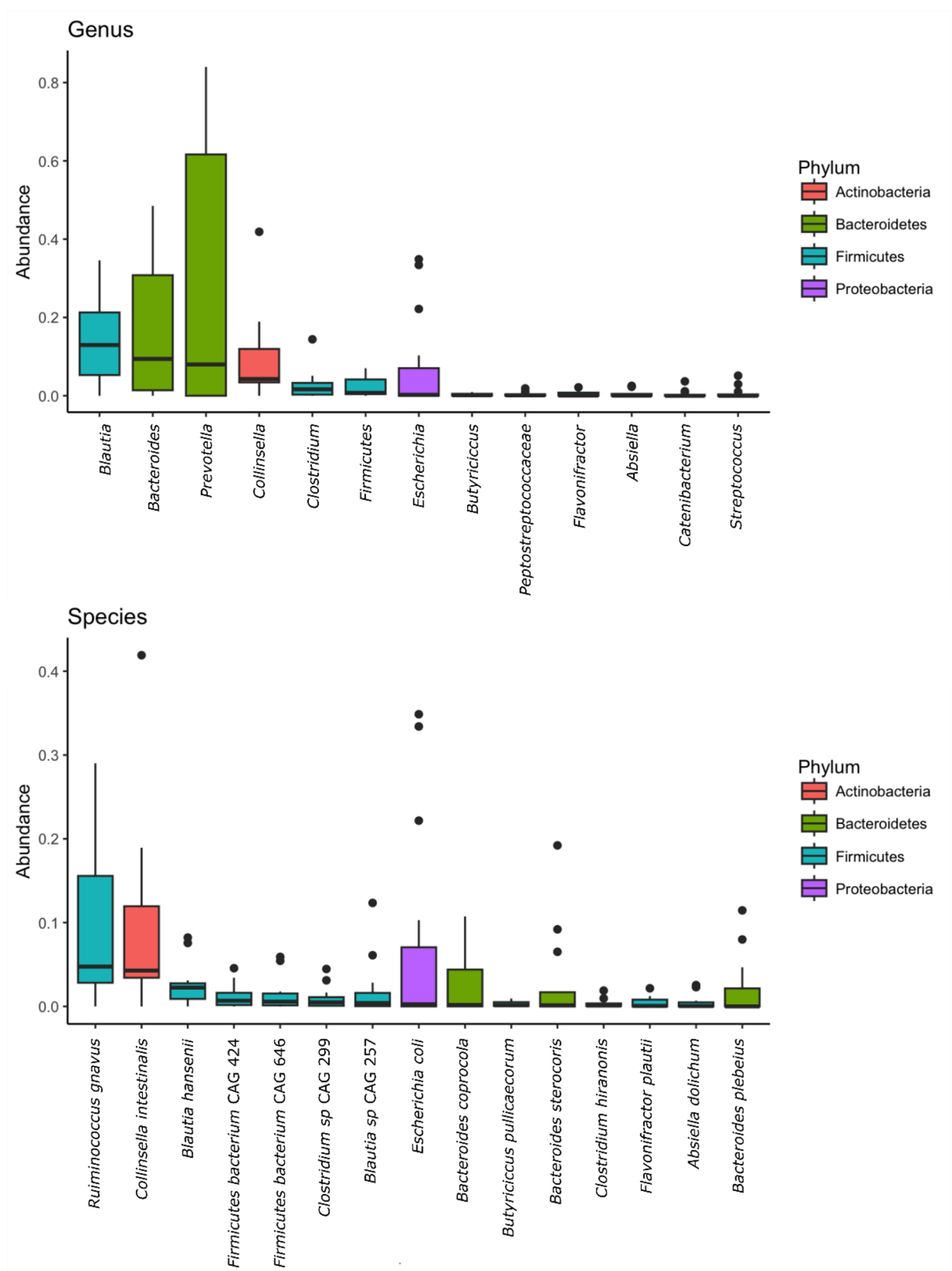


Fig. 2 Summary of the relative abundance of the most prevalent bacteria in BL at genus and species taxonomic levels. The top panel shows the relative abundance (Y axis) of most prevalent bacteria at genus level (X axis). The bottom panel shows the relative abundance (Y axis) of most prevalent bacteria at species level (X axis). Phylum is indicated by color

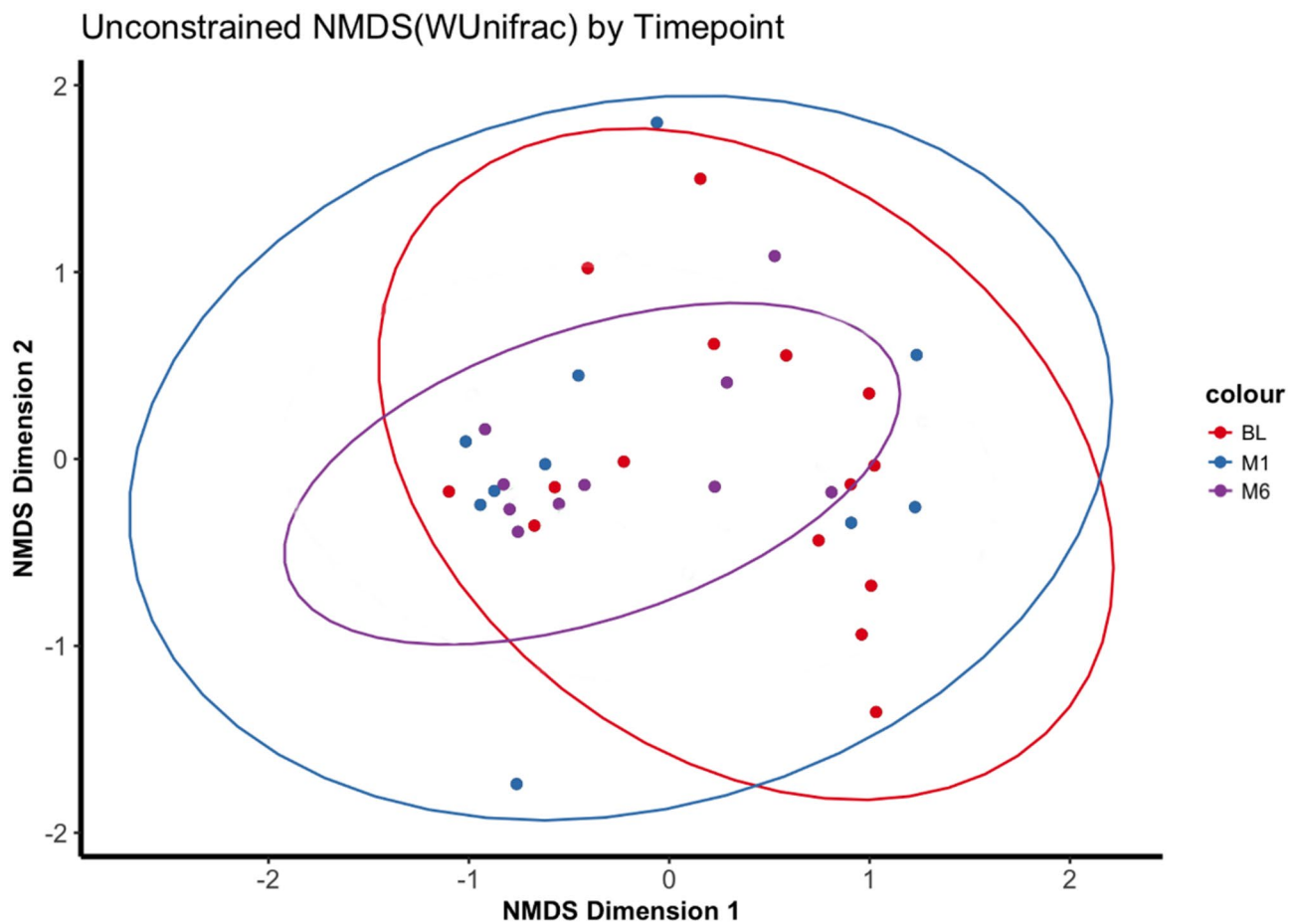


Fig. 3 Projection of microbiome communities by non-metric dimensional scaling (NMDS) of each dog before and after treatment. The axis and units are arbitrary. Colors depict time point of sampling bar plots corresponding to pre-treatment (BL), and one- and six-months post-treatment (M1 and M6). Ellipses (ordiellipse) function) collects the area where most samples fall within a timepoint

to the combined treatment for a month (PERMANOVA p -value = 0.426). Moreover, individual dog microbiome diversity represented 61% of the overall diversity.

Similar results were obtained after long-term administration of allopurinol, when samples before treatment and six months after initial treatment were analyzed as pairs (BL vs. M6). As shown in Fig. 4b, there were no significant differences in taxonomy (Wilcoxon p -value = 0.48) or gene (Wilcoxon p -value = 0.19) sample diversity (alpha diversity) before treatment and after six months of allopurinol treatment. A comparison of the microbiome communities (Bray-Curtis dissimilarity) before and after 6 months of treatment with allopurinol showed no statistical difference (PERMANOVA p -value = 0.11).

Individual species present in the M1 and M6 communities were compared with the same species present in the BL to identify differentially enriched or depleted taxa due to treatment. Linear discriminant analysis, using LEfSe [32], was performed, identifying possible enrichments of *Bifidobacterium pseudocantenum*, *Bifidobacterium longum*, *Collinsella tanakaei*, *Bifidobacterium pullorum*

and *Allisonella histaminiformans* in M1 and *Prevotella copri* and *Slackia piriformis* in M6. The method detected a decrease in *Erysipelatoclostridium ramosum* in BL. After significance testing and correcting for multiple testing (Kruskal-Wallis with Bonferroni multiple testing correction, p -value > 0.005), no hits were statistically significant. Results were confirmed with microbiome multivariable association with linear models in MaAsLin2 [34].

Discussion

This study aimed to investigate the impact of antileishmanial treatment on the gut microbiome composition with 10 sick dogs of leishmaniosis from three clinical facilities (Spain, Italy, and Portugal). Treatment adherence and clinical follow up were compiled for the duration of the study (six months). DNA extracted from fecal samples was sequenced using undirected metagenomics, an approach that allows the simultaneous detection of all DNA components of the microbiome (viruses, bacteria, archaea, or eukaryotes) compared with amplicon sequencing of marker genes (e.g., 16S) [35].

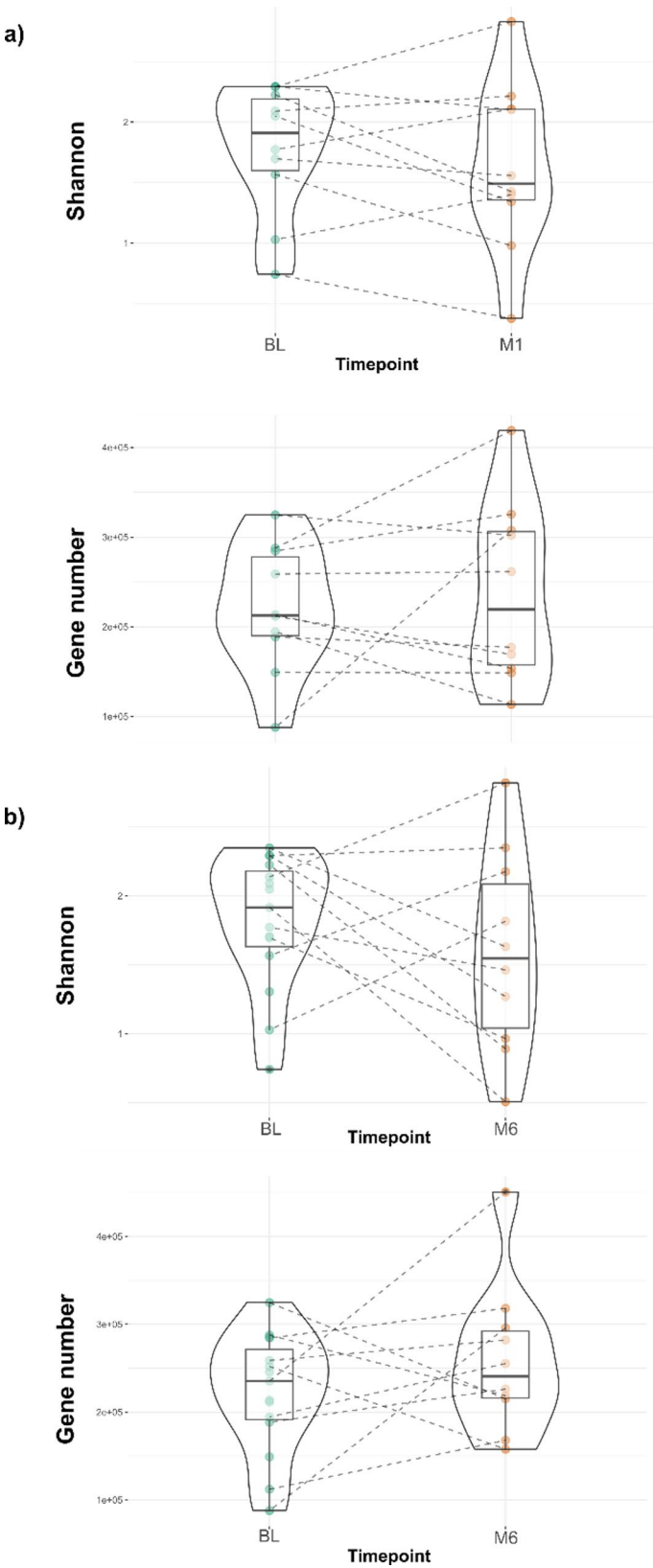


Fig. 4 Comparison of taxonomic diversity and functional diversity before treatment and after one month of treatment. Panel **(a)** Gut microbiome diversity is compared before (BL) and after one month of treatment (M1) for each dog using Shannon index (top panel) to estimate taxonomic diversity and gene number (bottom panel) to estimate functional diversity. Panel **(b)** Gut microbiome diversity is compared before (BL) and after six months of treatment (M6) for each dog using Shannon index (top panel) to estimate taxonomic diversity and gene number (bottom panel) to estimate functional diversity

Microbiome composition

The taxonomic composition of the gut microbiome in *Leishmania*-infected dogs over six months revealed remarkable individual variability, despite the presence of core taxa shared across the cohort. Across the 10 dogs sampled, the gut microbiome was dominated by members of the *Prevotella*, *Collinsella*, *Bacteroides*, and *Blautia* genera, which is consistent with previous studies indicating the role of these genera in gut of healthy dogs [36, 37]. The canine healthy gut bacteria composition is rich, strongly represented by *Prevotella*, *Fusobacterium*, *Clostridium* (sensu lato), *Lactobacillus*, *Streptococcus*, *Escherichia/Shigella*, *Blautia*, *Ruminococcus*, *Rombustia* and *Turicibacter* genera [36, 37]. Their relative abundance or presence in the fecal tract may depend on multiple factors, including age or feed [37]. As healthy dogs age, relative abundance of *Prevotella copri*, *Alloprevotella rava* and *Bacteroides coprocola* decrease, meanwhile *Escherichia coli* increases. Similarly, healthy dogs that do not consume kibble regularly have the relative abundances of *Bacteroides vulgatus*, *Caballeronia sordidicola*, *Enterococcus faecium*, *Erysipelatoclostridium ramosum*, *Blautia* sp. and *Clostridium* sp. increase, whereas that of healthy dogs that consume kibble regularly have an increase in the relative abundance of *Collinsella intestinalis*, *Turicibacter sanguinis*, *Megamonas funiformis*, *Holdemanella biformis*, and *Prevotella copri* [37]. In this study, such trends were not observed, as gut microbiome composition at pretreatment (BL) had no significant differential enrichment in bacterial relative abundance due to sex, breed, age, neutered status, country of origin, lifestyle, habitat, possible cohabitations, or type of diet (LEfSe and MaAsLin2 analysis). In fact, using Bray-Curtis dissimilarity (Fig. 3), the primary driver of microbial diversity was the individual dog, accounting for 52% of the observed diversity. This finding is consistent with findings from both canine and human microbiome studies, where individual host factors (e.g., genetics, diet, and environment) have been shown to play a significant role in shaping microbial communities [38–40].

In *Leishmania*-infected models, microbiome changes have been linked to *Leishmania*-infected mice [13]. OcB/Dem breeds have been shown to be resistant to *Leishmania* infection, with positive associations with *Lactobacillaceae* and *Clostridiales*, *Peptococcaceae*, *Tannerellaceae* and *Burkholderiaceae*. Moreover, CsS/Dem breeds have been shown to be susceptible to *Leishmania* infections with positive associations with *Rikenellaceae*, *Deferribacteriaceae* and *Firmicutes* [13]. These results do not translate to other rodent models, such as Syrian hamsters, where no significant microbiome alterations could be identified due to a *Leishmania* infection [15]. Similarly, in *Leishmania*-infected dogs (sick or healthy) *Adlercreutzia*, *Prevotella*, *Clostridium*, *Enterococcus*,

Dorea, *Megamonas*, *Roseburia* and *Escherichia* genera are differentially abundant in healthy dogs compared to *Leishmania*-infected dogs [16]. In humans with visceral leishmaniasis, changes in microbiome composition have been reported in *Ruminococcaceae* and *Gastranaerophilales* between healthy and sick humans [17]. In this study, focusing on gut microbiome composition at pretreatment (BL), no members of *Peptococcaceae*, *Deferribacteriaceae*, *Adlercreutzia* or *Roseburia* were identified. The *Prevotella*, *Clostridium* and *Enterococcus* genera have many species present in the set of samples (40, 140 and 171 respectively), whereas the *Escherichia*, *Megamonas* or *Dorea* genera, despite being present and having several species representatives (20, 10 and 10 respectively) their species diversity is lower than *Prevotella*, *Clostridium* and *Enterococcus* genera. The relative abundance of these genera seems to be correlated with their diversity, having *Prevotella*, *Clostridium* and *Enterococcus* genera a higher relative abundance than *Escherichia*, *Megamonas* or *Dorea* genera.

Furthermore, pretreatment (BL), *Prevotella copri* and *Collinsella intestinalis* were prominent members of the gut microbiome, with the former being one of the most abundant species (Figs. 1 and 2). These genera are often linked to the digestion of complex carbohydrates and fibers, indicating their potential importance in maintaining gut homeostasis [41], even in the context of a chronic infectious disease such as leishmaniasis. Moreover, the dominance of *Blautia* and *Bacteroides* is notable because these genera are typically associated with anti-inflammatory properties and short-chain fatty acid (SCFA) production [42]. The high prevalence of these taxa, particularly *Blautia*, which is linked to SCFA production, such as butyrate, suggests that even when dogs are infected with *Leishmania*, their gut microbiomes retain key microbial groups important for gut barrier function and immune modulation [43]. Interestingly, several minor bacterial genera, including *Butyricicoccus*, *Flavonifractor*, *Absiella*, and *Catenibacterium*, presented high variability in relative abundance across samples (Figs. 1 and 2). These genera, particularly *Butyricicoccus* and *Flavonifractor*, are known SCFA producers, which play crucial roles in gut health by modulating the host immune response and maintaining intestinal barrier function [44]. While their presence was not significant enough to drive large-scale microbiome changes, their sporadic occurrence in certain dogs might reflect individual differences in gut physiology or the immune response to *Leishmania* infection. Notably, that individual changes in the gut microbiome composition of specific taxa might not have a relevant impact on health status as a diverse microbiome does entail good functional redundancy, where the gut microbiome maintains its overall metabolic potential despite changes in microbial composition

[45]. *Collinsella intestinalis*, while not as abundant as *Prevotella*, displayed a higher prevalence, suggesting that this taxon may have a more consistent, albeit lower-level, role in the microbial community across different dogs, possibly contributing to maintaining metabolic functions over time, even in the presence of infection. The presence of *Escherichia coli* in 33% of the samples, without any clear association with the variables studied, is an intriguing finding. *Escherichia coli* is commonly associated with inflammation, especially in conditions such as inflammatory bowel disease in humans [46]. However, in this case, its sporadic appearance suggests that it may not play a central role in the microbiome changes associated with *Leishmania* infection or antileishmanial treatment, possibly reflecting individual variations in gut health or transient colonization by opportunistic pathogens.

Overall, these findings suggest that while *Leishmania* infection and its treatment do not dramatically alter the gut microbiome of infected dogs, there is substantial individual variability in microbiome composition. This variability may explain the observed resilience of the gut microbiome to infection and treatment and aligns with the increasing understanding of individual-specific microbiome responses in chronic diseases [47]. Future studies should explore the functional consequences of these microbial dynamics, particularly the role of SCFA-producing bacteria in modulating the immune response during chronic *Leishmania* infection.

Microbiome alteration due to antileishmanial treatment

The comparative analysis between baseline (BL) and post-treatment time points (M1 and M6) aimed to identify any significant shifts in microbiome composition due to the administration of meglumine antimoniate and allopurinol (M1) and long-term administration of allopurinol (M6). Our results indicate that the overall microbial diversity, both alpha diversity (Shannon index and gene number) and beta diversity (Bray-Curtis dissimilarity and weighted UniFrac), remained largely unchanged throughout the study.

Although the number of dogs included in the present study was not very large, the absence of statistically significant microbiome changes over time ($p\text{-value} > 0.05$) suggests that the gut microbiome changes in these *Leishmania*-infected dogs could not be linked to any common cause during the study. These findings suggest that the combined therapy (M1) or long-term single therapy (M6) does not induce significant changes in the microbiome communities during treatment.

Small enrichment trends, albeit not significant, were identified in M1 for *Bifidobacterium pseudocatenulatum*, *Bifidobacterium longum*, *Collinsella tanakaei*, *Bifidobacterium pullorum* and *Allisonella histaminiformans* and *Prevotella copri* and *Slackia piriformis* in M6. Small

depletion trends, albeit not significant, were identified in the BL treatment for *Erysipelatoclostridium ramosum*. These results could be interpreted either as false positives from LefSe, as hits disappeared after multiple testing correction and were not present in MaAsLin2 analysis, or hits with low statistical power due to the small sample size.

The microbiome communities seem to be unaffected by the given treatment, with only uncertain evidence indicating small enrichments in *Bifidobacterium*, *Collinsella*, *Allisonella*, *Prevotella*, and *Slackia* genera. These taxa are commonly found in healthy microbiomes, suggesting a possible neutral effect of the treatment to the gut microbiome composition, which may be crucial for maintaining gut health and overall homeostasis during disease and recovery (Kinross et al., 2011). Drugs used to treat *Leishmania*, such as antileishmanial agents, may have similar effects.

Non-antibiotic drugs have been shown to alter microbial communities, potentially disrupting the balance of the gut microbiome, although the exact mechanisms remain largely unknown (Maier et al., 2018). However, studies examining the direct impact of antileishmanial treatments on the microbiome are limited, highlighting the need for such studies. One recent study investigating the effects of miltefosine in *Leishmania infantum*-infected Syrian hamsters reported that the drug did not significantly alter the gut microbiome composition (Olías-Molero et al., 2023). These findings suggest that miltefosine may not impact microbial populations in this model, yet possible effects may exist for other drugs, such as meglumine antimoniate or allopurinol or in a different disease models or hosts, such as dogs or humans. In the present study, meglumine antimoniate and allopurinol treatment had no significant effect on microbiome populations before and after treatment in a relevant model and host (dogs) with clear implications for leishmaniosis treatment and *Leishmania* management. This study promotes that standard antileishmanial treatments can be administered without major concerns regarding the disruption of gut microbiome, which is critical for maintaining the overall health of infected dogs. However, owing to the low number of enrolled dogs in the longitudinal study, further research would be required to validate the results. Additionally, the significant individual variability observed in the microbiome composition obscures the possible role of individual treatment plans (i.e., diet, probiotics or exercise). Thus, future leishmaniosis trials should try to evaluate the effect of individualized treatment plans for recovery of dogs with leishmaniosis [2].

Conclusion

In conclusion, although the number of dogs included is low, this study provides evidence that the recommended combined treatment of meglumine antimoniate and allopurinol during the first six months does not significantly alter the gut microbiome composition of dogs with leishmaniosis. Moreover, most of the observed changes in the gut microbiome are individual specific, highlighting the unique and dynamic nature of microbiome gut communities in dogs. However, due to low possible enrollment due to the study constraints, further research should be considered to discard any minor change or contribution during the above-mentioned treatment.

Abbreviations

Bp	Base pairs
BL	Baseline (pre-treatment)
ELISA	Enzyme-linked immunosorbent assay
IFAT	Immunofluorescent Antibody Test
M1	Month 1 post initial treatment
M6	Months 6 post initial treatment
PCR	Polymerase chain reaction
SCFA	Short-chain fatty acid

Supplementary Information

The online version contains supplementary material available at <https://doi.org/10.1186/s42523-025-00447-3>.

Supplementary Material 1: Additional file 1: **Table S1:** Abundance (counts) and taxonomy information of each sample in the study by species. **Table S2:** Abundance (counts) and taxonomy information of each sample in the study by genera. **Table S3:** Abundance (counts) and taxonomy information of each sample in the study by phyla

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Author contributions

JMC has written the manuscript and elaborated the figures. MC performed the laboratory preps and sample analysis. MNJ analyzed the sequencing data and performed the microbiome study. ROL, GO and XR conducted the sample collection from their veterinary practices and provided clinical data and questionnaires. OF, LF, JM and XR designed the study and directed its steps. All authors contributed to the writing and revision of manuscript.

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Data availability

The study sequencing data is available under NCBI BioProject PRJNA1199800 under BioSample records SAMN45888860, SAMN45892024 - SAMN45892052 and demultiplexed reads under SRR31760315- SRR31760344.

Declaration

Ethical approval and consent to participate

Dog owners' written consent was obtained for fecal sampling and access to veterinarian records, if required. A basic questionnaire was given for dog lifestyle data.

Consent for publication

All authors have given their consent for the publication of this study in its present form.

Competing interests

The authors declare no competing interests.

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