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Microbiome as a Diagnostic Tool: Identifying Resistance to *Haemonchus contortus* in Goats through Metagenomic Profiling

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Abstract

Gastrointestinal nematodes (GINs), particularly *Haemonchus contortus* (*H. contortus*), pose significant challenges to small ruminant health and productivity, with increasing resistance to commercial anthelmintics. Conventional diagnostic techniques, such as fecal egg counts (FEC) and larval cultures (LC), are labor-intensive, time-consuming, and require technical expertise, limiting their practicality in field settings. There is an urgent need for faster, more accessible diagnostic tools to inform treatment decisions and improve parasite management strategies. In this study, we hypothesized that changes in microbiota, specifically shifts in microbial richness, community composition, and consistency, can serve as reliable biomarkers for infection and resistance. To test this hypothesis, we employed a metagenomic approach to characterize microbial communities in Alpine wethers based on their GIN infection status. Animals were divided into four groups: uninfected controls, infected-only, infected and treated with zoledronic acid (a bisphosphonate, previously believed to act as an antibiotic), and infected and treated with a neutralizing antibody targeting $\gamma\delta$ T cells. Metagenomic analyses of tissue samples revealed distinct microbial signatures associated with host susceptibility or resistance. Infection with *H. contortus* was linked to inflammatory microbial profiles, which varied depending on the treatment and infection status. Our findings suggest that inflammation-associated microbial taxa could serve as indicators of parasitic infection, supporting the development of more precise, data-driven diagnostic tools for early detection of haemonchosis. Additionally, by identifying microbiota-based markers of resistance, this research paves the way for more sustainable parasite control strategies, including microbiota manipulation and fecal microbiota transplantation. Overall, metagenomics provides a robust framework for investigating host-pathogen interactions and uncovering novel approaches to combat parasitism in livestock.

Keywords

Metagenomic, Gastrointestinal Nematodes, Microbial Flora, *Haemonchus contortus*, Host-Parasite Interaction

1. Introduction

Internal parasites significantly challenge small ruminant production and efficiency [1] [2]. Resistance to commercial anthelmintics limits the ability to control parasites through this method alone [3]. Like other ruminants, goats are susceptible to gastrointestinal parasite (GIN) infections [4]-[5]. The host microbiome can be altered by either successful or unsuccessful infections [6] [7]. Current diagnostic methods for detecting resistance primarily rely on conventional techniques such as fecal egg counts (FEC) and larval cultures (LC) [2] [8]. Assessing infection status requires time, cost, and skilled personnel [2]. Faster, more user-friendly field-based diagnostic tools for identifying infection or resistance are gradually emerging [2] [7]; however, if available, they would significantly improve the ability to detect GIN infections in goats. Therefore, this study offers a solution to this missing option. We hypothesize that numerous parameters will be included as additional data points, providing a more precise means to identify infection or health based on similarity to uninfected control microbial flora parameters. This detection method identifies resistance or susceptibility in wethers when involving *H. contortus*. This study targets explicitly identifying *H. contortus*' effects on wethers based on differences in microbial flora between treated and non-treated control wethers.

We present an examination of two methods for inducing cytotoxic activity against targets: Zoledronic acid (ZA), a bisphosphonate that activates $\gamma\delta$ T cells [9]-[11], and a neutralizing antibody (AB) against $\gamma\delta$ T cells. While ZA doesn't primarily target parasitic worms directly, it is highly effective in mediating immunopathology during chronic inflammation without compromising protective immunity to infection [11]. AB has demonstrated that $\gamma\delta$ T cells play significant roles in crosstalk between innate and adaptive immunity, as these cells actively regulate the adaptive immune response through interactions with antigen-presenting cells, such as dendritic cells [12]. We conducted a study investigating the effects of *Haemonchus contortus* (*H. contortus*) infection on Alpine wethers (young castrated male goats), including the potential impacts of ZA and AB on identifying or initiating resistance to the parasite.

H. contortus, also known as the barber pole worm, is a parasite that feeds by forming necrotic tissue. We hypothesize that these formations significantly alter the available environmental niches within the host, leading to localized and broader changes in the diversity and composition of the microbiome [13]. The microbiome, a community in the subjects we examined, is used to determine the relative proportions of microbial community members, the richness of these communities, and the evenness of their distribution in both GIN-infected and uninfected hosts.

2. Materials and Methods

2.1. Animals and Treatments

We conducted investigations of the microbiomes in blood, mesentery lymph nodes, abomasum lymph nodes, abomasum, abomasum fluid, fecal, and rumen fluid to identify significant differences in the subjects' relative populations of microbial flora.

Alpine wethers (n = 40) (114.2 ± 0.92 days of age and 19.4 ± 0.33 kg body weight (BW) at the initiation of the study) that had been raised in indoor pens at the Langston University AIGR farm were used. All subjects were checked for fecal egg counts (FEC) and drenched with Cydectin, an anthelmintic that removes existing parasites. The wethers were allocated randomly to four groups of 10 animals each, and two or three animals from each group were placed in one of the four pens. As shown in **Table 1**, treatment groups and types were prepared for the study. All wethers were allowed to acclimatize to pens and feeders for one week. Subjects were fed 500 g of ground prairie hay (50%) and alfalfa (50%) daily.

Table 1. Treatment groups. Four groups of ten subjects each (1 - 4) for two collection time points (7 dpi and 21 dpi): + means positive /present, and − means negative/not present for the respective columns. Acronyms: NI: No infection, IO: Infection only, AB: Infected and treated with AB, and ZA: Infected and treated with ZA.

Group	Treatments		
	L3 *H. contortus* infection	Zoledronic acid	$\gamma\delta$T depletion
NI	−	−	−
IO	+	−	−
ZA	+	+	−
AB	+	−	+

Samples were obtained from each goat and time point from blood, mesentery lymph node, abomasum fluid, abomasum, abomasum lymph node, fecal, and rumen fluid samples on day seven and 21 days post inoculation (dpi) (n = 280).

The AB injection was administered intravenously on the first (1) day before the *H. contortus* infective larvae infection (L3; hatched and isolated from feces collected from LU goats). ZA was administered intravenously 7 days before and on 0, 7, and 14 dpi—initially, all wethers except those in Group I were given 10,000 *H. contortus* L3 by gavage.

2.2. DNA Extraction, Library Preparation, Sequencing, and Sequence Processing

Nucleic acid sample extractions and libraries were prepared from the following tissues: Abomasum (Abo), abomasum lymph nodes (ALN), mesentery lymph nodes (MLN), abomasum fluids (AF), rumen fluids (RF), fecal (Fec), and blood (Blood). Barcode placement and third-party Swift Biosciences 16S rRNA NGS Illumina MiSeq instrument DNA sequencing at 30X coverage were completed on the company's site. Out of 280 samples, 217 samples collectively passed Quality assurance and quality control (QA/QC). FastQC was used for adaptor trimming of the pairwise raw sequence data obtained. The reference genome (*Capra hircus*) used to separate the microbial flora was obtained from the National Center for Biotechnology Information (NCBI). Sequences (raw fastq pairwise sequences) were aligned using the default Burrows-Wheeler alignment tool (BWA) parameters, filtered and normalized, and analyzed for bacterial flora using MiniKraken [14] [15] on a proprietary suite of software by Partek Flow (Partek Flow: St. Louis, Missouri, USA) [16] [17].

QA/QC analyses were used for 7dpi (n = 19) and 21 dpi (n = 20) samples that underwent microbiome analyses using MiniKraken [14] [15]. After classifying the sequences into microbial flora and performing their taxonomic classification, there were 55,739 OTUs at the species level from 217 samples. The raw sequence data were uploaded to the National Center of Biotechnology Information (NCBI) and have been released to the public since September 2020.

3. Results

The microbial community composition within and between tissue samples indicates the presence of parasite infection. Comparing the pairwise fastq sequence data collected from different samples according to each treatment helps identify shared patterns of OTU presence. **Supplementary Table S1** presents the Alpha Diversity Report, including all samples used in this study that failed QA/QC. **Supplementary Table S2** lists the genera and species identified in the treatment comparisons. The only comparison without significant differences in genus and species was Infection only versus Infection ZA. The table includes all treatment comparisons that resulted in more than one genus and species. **Supplementary Table S3** shows the genera and species identified in an additional comparison of treatment types, specifically comparing Infection only to Infection-AB, highlighting all comparisons with more than one genus and species.

Figure 1(A) depicts Beta diversity using the Bray-Curtis coefficient to detect differences in community structure caused by interventions such as diet or antibiotics. ANOVA tests group differences, for example, between healthy and diseased cohorts, and serves as a basis for ordination plots. As shown, the plot is very complex in illustrating relationships between samples, explaining the detailed patterns presented in the tables.

Figure 1(B) illustrates Beta diversity with the Jaccard index, a metric that measures the presence or absence of species or OTUs between samples or communities. It assesses how similar or different two communities are in terms of species composition. The Jaccard index is a simple, presence/absence-based metric that quantifies shared species between communities. The figure also reveals complex relationships among the samples from the studied subjects.

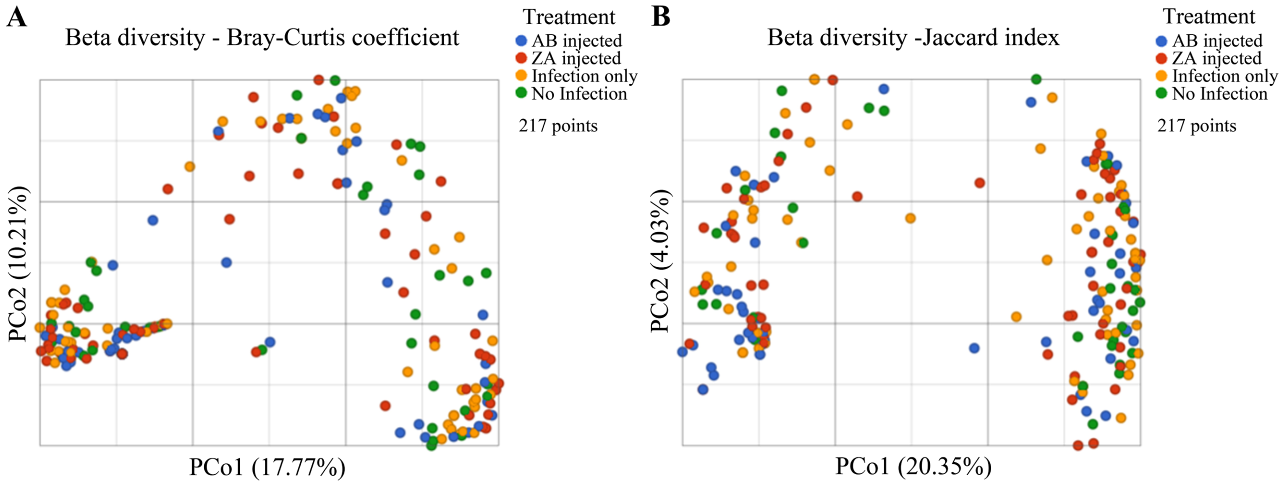

Figure 1. (A) Beta diversity-Bray-Curtis coefficient. (B) Beta diversity-Jaccard index.

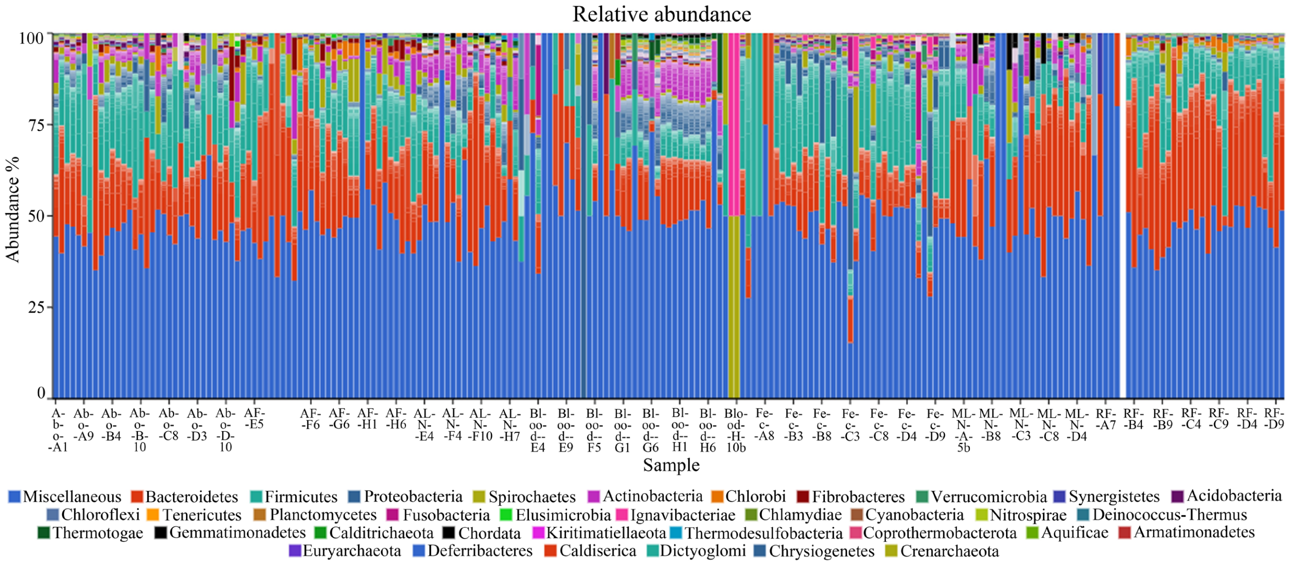

Figure 2. Relative Abundance of the total microbiome study comparing microbial flora from blood (blood), abomasum samples (Abo), abomasum fluid samples (AF), abomasum lymph node samples (ALN), fecal samples (Fec), mesentery lymph node samples (MLN), and rumen fluid samples (RF) from four treatments (NO, IO, ZA, and AB).

The sample name, treatment, days post inoculation (dpi), and classified reads for species with no rank corresponding to the microbial flora were placed in a spreadsheet following the Minikraken results. The relative abundance of microbial flora was placed in bar charts in Figure 2. The relative abundance of the total microbiome was studied by comparing microbial flora from four treatments (NO, IO, ZA, and AB).

When the data were analyzed based on all the parameters related to identified microbial flora and differential analysis (Kruskal-Wallis), the following figures of results show vertical lines based on (default at log2 fold changes of −2 and +2), indicating the magnitude of change considered biologically meaningful. The horizontal line (default at P-value = 0.05, corresponding to −log10 (0.05) ≈ 1.3) marked the threshold for statistical significance. Infection only versus no infection (**Figure 3**) had two microbial flora species that were downregulated (*Caldithrix* abyss and *Ruminococcus* albus). These two showed significantly different downregulation when analyzed in this manner. All other points defined by microbial flora were either Inconclusive (28) or non-significant (70). When selecting specific microbial flora species for comparison on a scatter plot (*Aminobacterium colombiense* versus *Aequorivita sublithincola*), a Heatmap, and the Kruskal-Wallis analysis plot comparing Infection only versus no infection, **Figure 3** resulted. When comparing *Aminobacterium colombiense* versus *Aequorivita sublithincola* presence in the treatments, 161 points were identified, with four points appearing that were significant differences for infection-only treatments. Differential analysis using Kruskal-Wallis of the comparison between subjects with samples infected by *H. contortus* versus those not infected at all. Two microbial flora species show significant differences in downregulation −log10 (P-value) (*Caldithrix* abyss and *Ruminococcus* albus) with a log2 (Fold change). A heat map of three select samples (Langston-AF-H4_S351, Langston-Fec-B10_S196, Langston-MLN-D4_366) is included in **Figure 3**, indicating a higher presence of *Prevotella ruminicola* across abomasum fluid and rumen fluid, and a low presence in the remaining tissues.

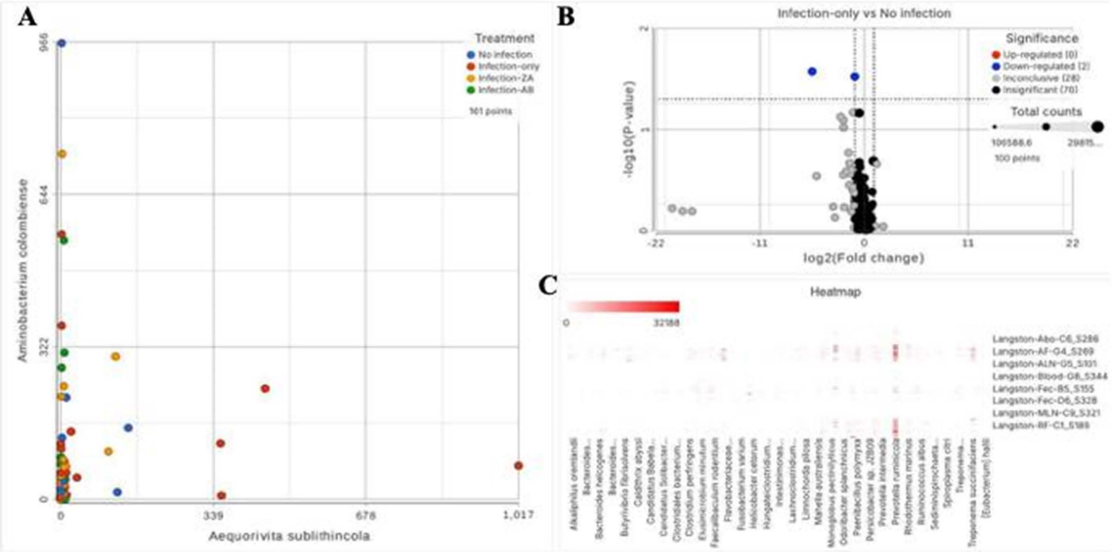

Figure 3. (A) A scatter plot comparing *Aminobacterium colombiense* versus *Aequorivita sublithincola*; (B) Differential analysis using Kruskal-Wallis of the comparison between subjects that had samples that were infected by *H. contortus* versus those that were not infected at all; (C) A heat map of a select three samples (Langston-AF-H4_S351, Langston-Fec-B10_S196, Langston-MLN-D4_366).

Figure 4 shows a differential analysis using Kruskal-Wallis of the comparison between subjects not infected by *H. contortus* samples versus those infected but injected with zoledronic acid antibiotic (ZA). Four microbial flora species show significant differences in upregulation (*Caldithrix abyss*, *Bacteroides coprosuis*, *Alkaliphilus oremlandii,* and *Treponema caldarium*), out of 104 points. Forty points were Inconclusive, and 56 were not significant.

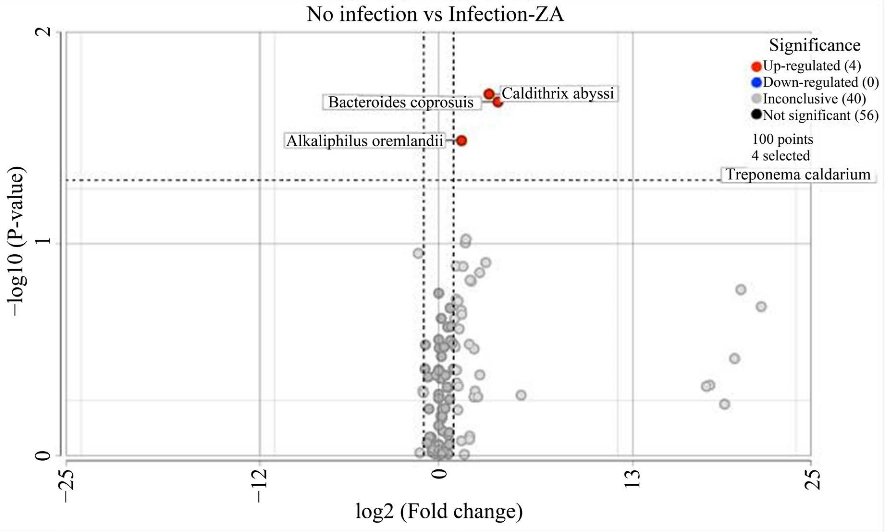

Figure 4. Differential analysis using Kruskal-Wallis of the comparison between subjects that had samples that were not infected by *H. contortus* versus those that were infected but injected with zoledronic acid antibiotic (ZA).

The comparison of Infection-ZA versus Infection AB injected resulted in no Up-regulated or Down-regulated microbial flora (points) as shown in **Figure 5**. 78 Inconclusive and 22 Insignificant points or microbial flora were identified.

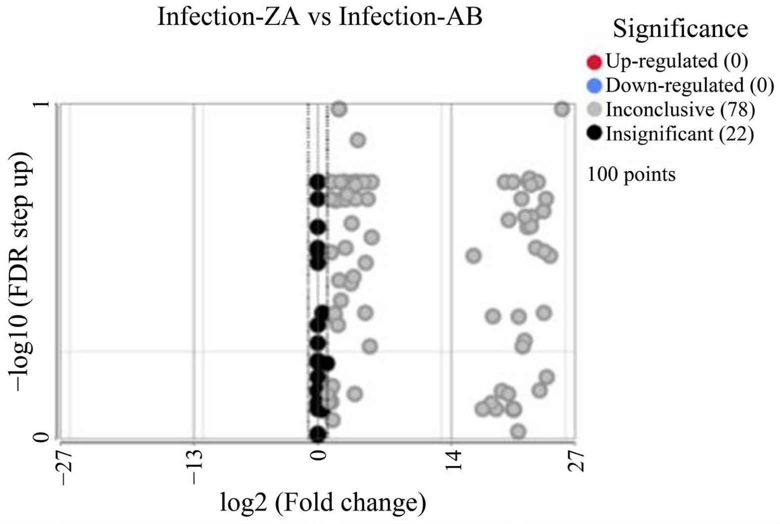

Figure 5. Differential analysis using Kruskal-Wallis of the comparison between subjects that were Infection-ZA versus Infection AB injected.

Figure 6 shows that comparing IO versus ZA resulted in no Up-regulated or Down-regulated microbial flora (points). 32 Inconclusive and 68 non-significant points or microbial flora were identified (Figure 6).

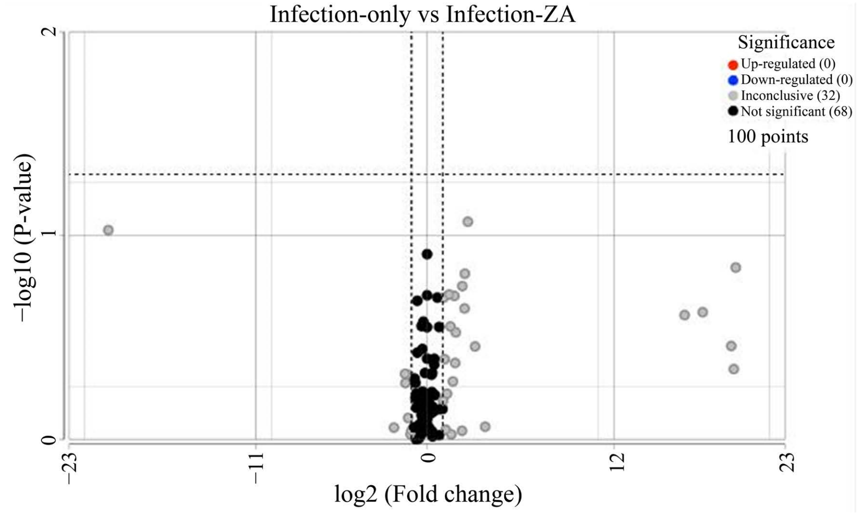

Figure 6. Differential analysis using Kruskal-Wallis of the comparison between subjects that were Infection-only versus Infection ZA injected.

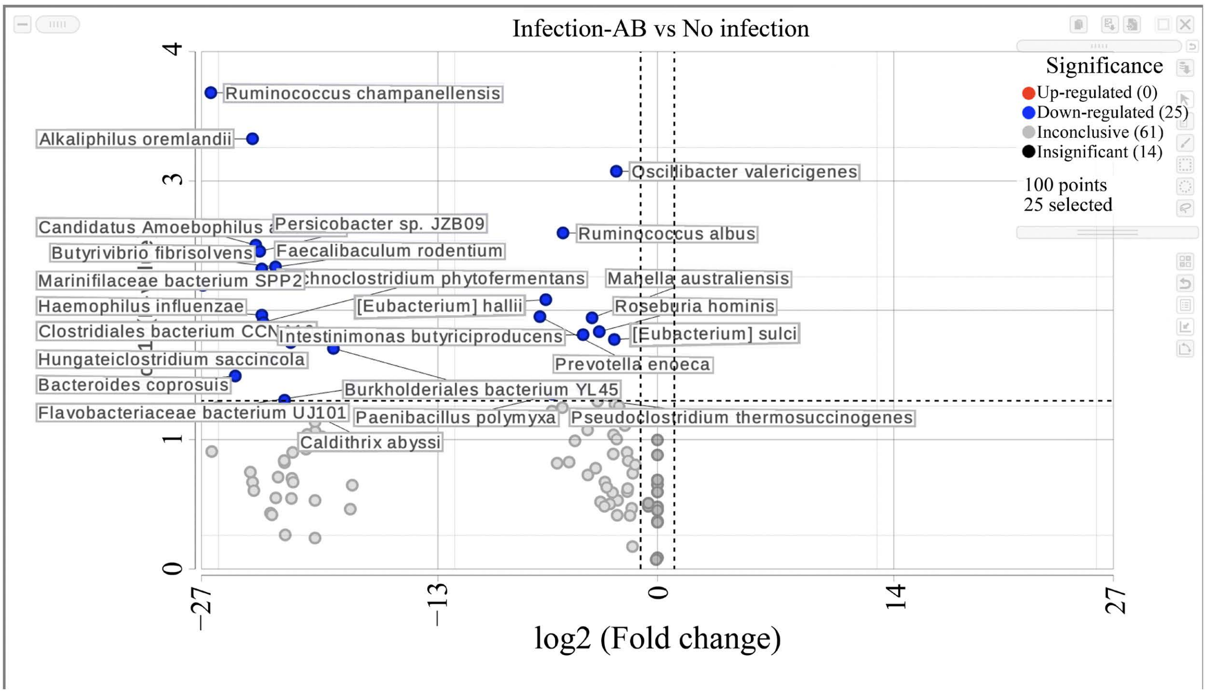

Figure 7. Differential analysis using Kruskal-Wallis of the comparison between subjects that were Infection-AB injected and those that were designated as No infection.

As shown in the Supplementary Table S2, comparisons with the most micro-bial flora identified that were significantly different belonged to the comparisons Figure 7: Infection AB vs No infection and Figure 8: Infection-only vs Infection-AB.

Figure 7 revealed 100 points representing 100 microbial flora present when comparing the treatments, Infection-AB injected and No infection. Results of the comparison showed 25 down-regulated significantly different microbial flora, 61 inconclusive microbial flora, and 14 insignificant microbial flora.

Figure 8 revealed 121 microbial flora when comparing the infection-only vs infection-AB injected treatments. Results of the differential equation showed significantly different 21 up-regulated microbial flora, 63 inconclusive microbial flora, and 16 insignificant microbial flora.

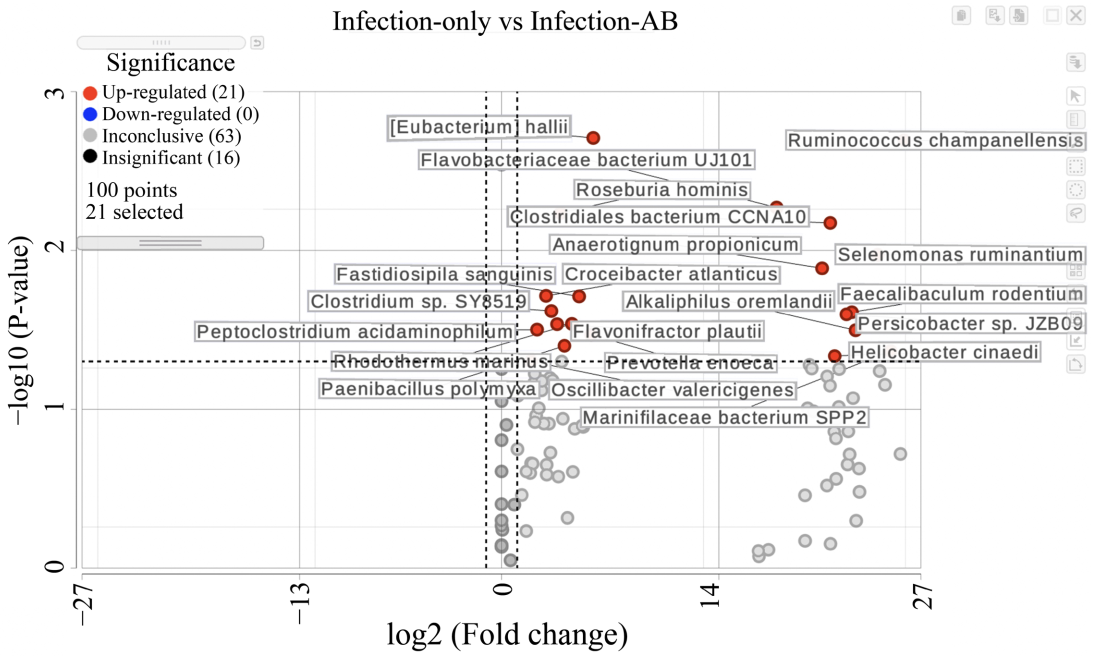

Figure 8. Differential analysis using Kruskal-Wallis of the comparison between subjects that were infection-only and infection-AB injected treatments.

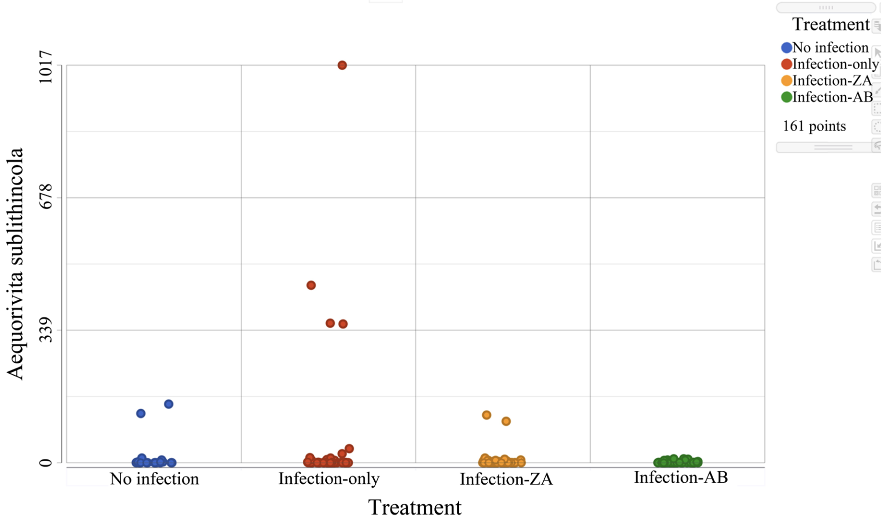

Figure 9. A scatter plot comparing the presence of *Aequorivita sublithincola* in treatments (No infection, Infection-only, Infection-ZA- ZA injected, and Infection-AB injected).

When observing a scatter plot such as the one depicted in **Figure 9**, there are 161 points in the comparison of the presence of *Aequorivita sublithincola* in the different treatments, with 4 points over the threshold of 339 for infection-only treatments.

4. Discussion

The microbiome is known to change constantly compared to the genome [18]. An earlier study [7] identified transcripts and the blood microbiome of Alpine goats in the host response to Infection with internal parasites, *H. contortus.* In the study, blood was examined, and the data obtained are included in this manuscript following patterns indicative of days post inoculation with *H.contortus* and different treatments, as described here.

Coefficient detection in community structure due to interventions was examined along with test group differences. The ordination plots also included Beta diversity-Jaccard indices, a metric for measuring beta diversity, explicitly focusing on the presence or absence of species or OTUs between samples or communities. They quantified how similar or different two communities are regarding their species composition. The Jaccard Indices were a straightforward, presence/absence-based beta diversity metric that quantified how much two communities share in species composition. As can be seen from the ordination plots, there were interrelated points between and among the different treatments, making defining the relationship to non-infected samples difficult.

However, this study identified many microbial flora as up- and down-regulated when comparing AB with either no-infection or infection-only types of samples. The study showed changes in the complexity and richness of microbial flora at varying degrees in certain goats after seven dpi. Patterns identifying similarity with non-infected subjects indicate possible resistance in goats by being inhabited with similar types and amounts of microbial flora, with an injection of AB. Features of microbial flora that differ across individuals include taxonomic composition and structure, abundance, and the rate at which the composition and structure change over time [9] [14]. Several recent studies have identified the impact that microbiota have on the immune system [16] [17] [19] [20].

This metagenomic study investigates two time points (7 days post-inoculation and 21 days post-inoculation) and the time course of immune responses based on the microbiome reactions of wethers after infection with *H. contortus*. As mentioned, ZA, a bisphosphonate that activates $\gamma\delta$ T cells [9]-[11], and AB were injected into goats inoculated with L3 *H. contortus*. In this study, we injected ZA due to its known effects on the immune system. We compared the subjects using ZA to determine if there would be an alteration in the microbial composition of hosts infected by *H. contortus* [10]. Similarly, AB treatment was used for the same examination: to identify if there would be a change in microbial composition after affecting the immune system of the wethers in the study. The significance of AB treatments is that they demonstrate activity independently of CD4 proteins, which also seem to be involved in parasite control [14] [21]-[23]. The effects of the treatments on the wethers were assessed by examining changes in operational taxonomic units (OTUs) or microbial species, defined in blood, mesenteric lymph nodes, abomasal lymph nodes, abomasum, abomasal fluid, fecal, and rumen fluid samples.

In this study, the results identified that particular microbial flora are down-regulated and or up-regulated depending on the type of treatment. The most significant amount of down-regulated or up-regulated microbial flora appeared in those subjects infected with *H. contortus* and treated with a neutralizing antibody (AB) against $\gamma\delta$ T cells. These subjects and subjects infected with *H. contortus* and treated with the antibiotic ZA (zoledronic acid) appear to resemble subjects not infected with the parasite. They seem to be able to provide resistance to the parasite based on the operational taxonomic units (OTUs) expressed through down-regulation or up-regulation, and results of microbial OTU presence in the type of treatment.

When examining some comparison types with other types of infection or lack thereof, there are identical Genus species identifications. For example, *Alkaliphilus oremlndii* was identified as present with significant differences in the comparison types: Infection AB vs. No infection, Infection only vs. Infection-AB, and No Infection vs. Infection-ZA. *Caldithrix abyss* was identified to be present with a significant difference in comparison types: Infection AB vs. No infection, No Infection vs Infection-ZA, and Infection-only vs No infection. Most of the OTUs included Infection-AB versus other infection/treatment combinations.

5. Conclusion

The presence of *H. contortus* has been said to effectively change microbial habitat and composition in the caprine abomasum [20]. This study found noticeable tissue differences, especially when comparing blood, fecal, and abomasum tissue samples. When examining treatments, AB-injected subjects appear to resemble subjects not infected by the parasite *H.* contortus more than other treatments. Using non-invasive methods to identify infestation is ideal for further solidifying the construction tools and techniques for combating parasite infestation. This study shows that this is possible. Furthermore, these results encourage the development of more environmentally friendly methods of combating parasites. Using a neutralizing antibody (AB) against $\gamma\delta$ T cells appears to assist in reducing *H. contortus* parasite infection based on metagenomic profiles.

Authors' Information

These authors contributed equally: Yonathan Tilahun, Haji Akbar, and Zaisen Wang.

Authors Contributions

Y. T. and Z. W. conceived and planned the experiments. Y. T. and Z. W. carried out the experiments. Y. T. and Z.W. contributed to sample preparation. Y. T. and H. A. contributed to the interpretation of the results. Y. T. and H. A. were involved in writing the manuscript. All authors, including H.A., provided critical feedback and helped shape the research analysis and manuscript. All authors have approved the manuscript.

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Ethical Approval and Consent to Participate

The treatment of animals is abided by the guidelines of Langston University, Institutional Animal Care and Use Committee (LUACUC) Approval # 2018-14. All experiments were performed according to relevant guidelines and regulations. Furthermore, the reporting in this manuscript is done according to ARRIVE guidelines.

Data Availability Statement

The metagenomic data have been deposited with links to BioProject accession number PRJNA612987 in the NCBI BioProject database (https://www.ncbi.nlm.nih.gov/bioproject/).

Disclaimer/Publisher’s Note

Conflicts of Interest

“The authors declare no conflict of interest.” “The funders had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript; or in the decision to publish the results.”

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Supplementary

Supplementary Table S1. Effect size measured by the Alpha Diversity Report. Shannon and Simpson Indices indicate the richness and diversity present in each sample.

Sample name	Treatment	Days post inoculation	Shannon index	Simpson index
Langston-Abo-A9	Infection-AB	7	2.57	0.91
Langston-Fec-C2	Infection-AB	21	4.54	0.98
Langston-MLN-C3	Infection-AB	21	0	0
Langston-Abo-C7	Infection-AB	21	3.65	0.97
Langston-Abo-B4	Infection-only	7	4.48	0.97
Langston-RF-B2	No infection	7	0	0
Langston-ALN-H1	Infection-only	21	4.32	0.98
Langston-Abo-B8	Infection-ZA	7	4.33	0.97
Langston-AF-G4	Infection-ZA	21	3.65	0.93
Langston-RF-C6	Infection-only	21	2.64	0.79
Langston-Blood-H5	Infection-only	21	3.68	0.96
Langston-AF-G3	Infection-AB	21	3.5	0.92
Langston-Abo-B10	Infection-AB	7	4.61	0.98
Langston-RF-D6	Infection-only	21	2.96	0.81
Langston-RF-C10	Infection-ZA	21	2.34	0.9
Langston-Abo-C9	Infection-AB	21	1.83	0.81
Langston-RF-B4	Infection-only	7	2.69	0.81
Langston-Fec-B9	Infection-ZA	7	4.39	0.97
Langston-RF-C4	Infection-ZA	21	3.16	0.9
Langston-ALN-H3	No infection	21	3.62	0.94
Langston-Fec-B2	No infection	7	4.32	0.97
Langston-ALN-G8	Infection-only	21	3.75	0.96
Langston-ALN-G10	Infection-ZA	21	3.56	0.96
Langston-MLN-B6	Infection-ZA	7	2.75	0.92
Langston-RF-B10	Infection-AB	7	3.3	0.89
Langston-ALN-H6	Infection-only	21	3.52	0.97
Langston-ALN-G4	Infection-ZA	21	2.42	0.89
Langston-RF-C7	Infection-AB	21	3.01	0.84
Langston-Blood-G6	Infection-only	21	1.87	0.78
Langston-Fec-D5	Infection-only	21	4.47	0.98
Langston-RF-B7	Infection-ZA	7	3.14	0.85
Langston-RF-C1	Infection-AB	21	1.41	0.6
Langston-Fec-C10	Infection-ZA	21	4.27	0.97
Langston-MLN-C8	Infection-only	21	2.63	0.91
Langston-AF-F6	Infection-ZA	7	2.98	0.83
Langston-Abo-B5	Infection-only	7	4.63	0.97
Langston-MLN-B8	Infection-ZA	7	3.56	0.96
Langston-ALN-E4	Infection-only	7	3.61	0.96

Continued

Langston-Blood-H8	No infection	21	3.27	0.92
Langston-Fec-A10	Infection-AB	7	4.13	0.95
Langston-AF-G6	Infection-only	21	3.29	0.88
Langston-AF-G10	Infection-ZA	21	0	0
Langston-MLN-C9	Infection-AB	21	2.14	0.86
Langston-AF-F7	Infection-ZA	7	3.23	0.86
Langston-AF-H6	Infection-only	21	3.74	0.94
Langston-MLN-D4	Infection-ZA	21	2.15	0.88
Langston-Abo-B3	Infection-only	7	4.29	0.97
Langston-Abo-C6	Infection-only	21	1.91	0.84
Langston-MLN-C2	Infection-AB	21	2.87	0.94
Langston-Abo-B7	Infection-ZA	7	4.35	0.96
Langston-Fec-B6	Infection-ZA	7	4.34	0.97
Langston-Fec-C5	Infection-ZA	21	4.38	0.97
Langston-Fec-D1	Infection-only	21	4.33	0.97
Langston-Fec-C7	Infection-only	21	4.25	0.94
Langston-ALN-G5	Infection-ZA	21	0	0
Langston-Fec-D8	No infection	21	3.08	0.84
Langston-AF-G1	Infection-AB	21	3.48	0.93
Langston-ALN-H5	Infection-only	21	3.98	0.96
Langston-Fec-B4	Infection-only	7	4.2	0.96
Langston-Abo-D5	Infection-only	21	1.1	0.67
Langston-Fec-C6	Infection-only	21	4.26	0.97
Langston-Fec-D7	No infection	21	4.27	0.96
Langston-MLN-C1	Infection-AB	21	1.1	0.67
Langston-Fec-C4	Infection-ZA	21	3.29	0.86
Langston-AF-H2	Infection-ZA	21	3.81	0.94
Langston-Fec-B3	Infection-only	7	4.6	0.98
Langston-AF-G2	Infection-AB	21	3.74	0.93
Langston-Abo-C8	Infection-only	21	4.64	0.98
Langston-ALN-G6	Infection-only	21	2.42	0.9
Langston-MLN-D2	Infection-ZA	21	2.71	0.91
Langston-ALN-G9	Infection-AB	21	0	0
Langston-AF-H3	No infection	21	3.81	0.93
Langston-Fec-C8	Infection-only	21	4.47	0.98
Langston-Fec-D2	Infection-ZA	21	4.4	0.98
Langston-MLN-D5	Infection-only	21	0	0
Langston-Fec-A5	Infection-only	7	2.21	0.88
Langston-Blood-H2	Infection-ZA	21	5.15	0.98
Langston-Fec-B7	Infection-ZA	7	4.39	0.97
Langston-Abo-A10	Infection-AB	7	1.39	0.75

Continued

Langston-Fec-B10	Infection-AB	7	4.22	0.94
Langston-Blood-G4	Infection-ZA	21	5.05	0.98
Langston-RF-A10	Infection-AB	7	0	0
Langston-RF-C5	Infection-ZA	21	3.13	0.88
Langston-Fec-C9	Infection-AB	21	0.69	0.5
Langston-Fec-A4	Infection-only	7	4.03	0.97
Langston-Abo-A4	Infection-only	7	4.62	0.98
Langston-Fec-B8	Infection-ZA	7	4.79	0.98
Langston-Fec-D9	No infection	21	4.64	0.98
Langston-MLN-A4	Infection-only	7	1.7	0.8
Langston-Blood-G3	Infection-AB	21	1.1	0.67
Langston-Blood-G7	Infection-AB	21	4.77	0.98
Langston-Blood-H1	Infection-only	21	4.01	0.97
Langston-MLN-C4	Infection-ZA	21	3.15	0.94
Langston-RF-A7	Infection-AB	7	0	0
Langston-RF-B3	Infection-only	7	3.38	0.88
Langston-AF-H4	Infection-ZA	21	2.18	0.86
Langston-MLN-B7	Infection-ZA	7	3.18	0.96
Langston-Abo-D1	Infection-only	21	3.71	0.97
Langston-Blood-G5	Infection-ZA	21	5.23	0.98
Langston-RF-A8	Infection-AB	7	0	0
Langston-RF-D5	Infection-only	21	3.37	0.86
Langston-AF-H5	Infection-only	21	4.18	0.96
Langston-MLN-D3	No infection	21	3.87	0.94
Langston-Blood-H6	Infection-only	21	5.05	0.98
Langston-RF-D7	No infection	21	2.47	0.73
Langston-Abo-D4	Infection-ZA	21	2.54	0.92
Langston-AF-G7	Infection-AB	21	3.66	0.93
Langston-MLN-C7	Infection-AB	21	1.04	0.62
Langston-RF-D8	No infection	21	3.05	0.84
Langston-MLN-C5	Infection-ZA	21	1.24	0.67
Langston-RF-D1	Infection-only	21	2.73	0.79
Langston-MLN-C6	Infection-only	21	3.22	0.96
Langston-RF-B9	Infection-ZA	7	3.6	0.93
Langston-Fec-D6	Infection-only	21	3.56	0.92
Langston-RF-B6	Infection-ZA	7	3.28	0.9
Langston-AF-F8	Infection-ZA	7	3.55	0.92
Langston-RF-C3	Infection-AB	21	3.19	0.87
Langston-RF-A6	Infection-ZA	7	0.69	0.5
Langston-AF-H7	No infection	21	3.63	0.93
Langston-Fec-B5	Infection-only	7	3.96	0.95

Continued

Langston-RF-C8	Infection-only	21	3.07	0.83
Langston-Abo-C10	Infection-ZA	21	0.69	0.5
Langston-Abo-D2	Infection-ZA	21	4.91	0.98
Langston-AF-H1	Infection-only	21	3.8	0.94
Langston-ALN-H2	Infection-ZA	21	4.24	0.98
Langston-Blood-H7	No infection	21	1.11	0.58
Langston-ALN-G3	Infection-AB	21	0	0
Langston-Fec-A8	Infection-AB	7	0	0
Langston-AF-F10	Infection-AB	7	3.3	0.9
Langston-AF-H9	No infection	21	3.65	0.94
Langston-RF-C2	Infection-AB	21	3.14	0.85
Langston-RF-D9	No infection	21	3.05	0.85
Langston-RF-C9	Infection-AB	21	3.41	0.89
Langston-Abo-B6	Infection-ZA	7	4.81	0.98
Langston-Abo-C1	Infection-AB	21	3.42	0.92
Langston-Abo-A1	No infection	7	4.64	0.97
Langston-AF-F5	Infection-only	7	2.38	0.75
Langston-RF-D2	Infection-ZA	21	3.01	0.88
Langston-ALN-F1	No infection	7	4.08	0.97
Langston-Blood-G10	Infection-ZA	21	4.93	0.98
Langston-AF-H8	No infection	21	3.44	0.85
Langston-RF-D3	No infection	21	3.36	0.88
Langston-Abo-B1	No infection	7	3.27	0.86
Langston-AF-G5	Infection-ZA	21	3.67	0.94
Langston-RF-D4	Infection-ZA	21	2.98	0.81
Langston-Abo-D3	No infection	21	4.75	0.97
Langston-Blood-G8	Infection-only	21	5	0.97
Langston-Blood-H9	No infection	21	0	0
Langston-Fec-A9	Infection-AB	7	0	0
Langston-Abo-A5	Infection-only	7	4.75	0.98
Langston-Abo-B9	Infection-ZA	7	3.72	0.97
Langston-Fec-D4	Infection-ZA	21	4.47	0.97
Langston-Blood-G9	Infection-AB	21	4.97	0.98
Langston-MLN-B5	Infection-only	7	1.79	0.83
Langston-ALN-H4	Infection-ZA	21	3.41	0.94
Langston-Abo-B2	No infection	7	4.43	0.96
Langston-RF-B5	Infection-only	7	3.71	0.94
Langston-Blood-G2	Infection-AB	21	5.01	0.97
Langston-Blood-G1	Infection-AB	21	4.31	0.98
Langston-Fec-C3	Infection-AB	21	2.53	0.72
Langston-MLN-B4	Infection-only	7	0.69	0.5

Supplementary Table S2. The Genus and species that were identified in the comparisons of treatment types. The only comparison with no Genus and species identified with significant difference was Infection only vs Infection ZA. This table shows all the comparisons of treatments that resulted in > 1 or more Genus and species.

Genus and species identified	Comparison type	Comparison type	Comparison type	Comparison type
Ruminococcus champanellensis	Infection AB vs No infection	Infection only vs Infection-AB	-	-
Alkaliphilus oremlndii	Infection AB vs No infection	Infection only vs Infection-AB	No Infection vs Infection-ZA	-
Oscillibacter valericigenes	Infection AB vs No infection	Infection only vs Infection-AB	-	-
Candidatus Amebophilus	Infection AB vs No infection	-	-	-
Faecalibaculum rodentium	Infection AB vs No infection	-	-	-
Ruminococcus albus	Infection AB vs No infection	-	-	Infection-only vs No infection
Marinifilaceae bacterium SPP2	Infection AB vs No infection	Infection only vs Infection-AB	-	-
Lachnoclostridium phytofermentans	Infection AB vs No infection	-	-	-
Mahella australiensus	Infection AB vs No infection	-	-	-
Haemophilus influenza	Infection AB vs No infection	-	-	-
Eubacterium hallii	Infection AB vs No infection	Infection only vs Infection-AB	-	-
Roseburia hominis	Infection AB vs No infection	Infection only vs Infection-AB	-	-
Clostridiales bacterium CNN A10	Infection AB vs No infection	Infection only vs Infection-AB	-	-
Intestinimonas butyriciproducens	Infection AB vs No infection	-	-	-
Eubacterium sulci	Infection AB vs No infection	-	-	-
Hungateiclostridium succinctly	Infection AB vs No infection	-	-	-
Prevotella enoteca	Infection AB vs No infection	Infection only vs Infection-AB	-	-
Bacteroides coprosuis	Infection AB vs No infection	-	No Infection vs Infection-ZA	-
Burkholderiales bacterium YL 445	Infection AB vs No infection	-	-	-
Flavobaacteriaceae bacterium U J1 01	Infection AB vs No infection	Infection only vs Infection-AB	-	-
Paenibacillus polymyxa	Infection AB vs No infection	Infection only vs Infection-AB	-	-
Pseudoclostridium thermosuccinigenes	Infection AB vs No infection	-	-	-
Caldithrix abyss	Infection AB vs No infection	-	No Infection vs Infection-ZA	Infection-only vs No infection

Supplementary Table S3. The Genus and species that were identified in the comparisons of treatment types. This table shows all the comparisons of treatments that resulted with > 1 or more Genus and species when Infection only was compared to Infection-AB.

Genus and species identified	Comparison type
Anaerotignum propionicum	Infection only vs Infection-AB
Selenomonas ruminantium	Infection only vs Infection-AB
Fastidiosipila sanguinis	Infection only vs Infection-AB
Croceibacter atlanticus	Infection only vs Infection-AB
Clostridium sp SY8519	Infection only vs Infection-AB
Peptoclostridium acidaminophilum	Infection only vs Infection-AB
Flavonifractor plautii	Infection only vs Infection-AB
Persicobacter sp. JZB09	Infection only vs Infection-AB
Rhodothermus marinus	Infection only vs Infection-AB
Prevotella enoeca	Infection only vs Infection-AB
Helicobacter cinaedi	Infection only vs Infection-AB
Paenibacillus polymyxa	Infection only vs Infection-AB
Marinifilaceae bacterium SPP2	Infection only vs Infection-AB