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Manuscript peer review sample work

The Impact of Drinking Water Sources on Gut Microbial Diversity in Canines

SAMPLE WORK

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Meta Description: Pubrica provides a peer review for an interesting investigation into the influence of drinking water sources on canine gut bacteria diversity.

The Impact of Drinking Water Sources on Gut Microbial Diversity in Canines

> **PEER REVIEW AUTHOR**
> Manuscript could benefit from further clarification and expansion in several areas to strengthen its scientific rigor and enhance the clarity of its findings

Abstract

The existence of gut microflora in the gastrointestinal tract (GIT) significantly influences antiviral defenses, digestion, and various other physiological aspects. Changes in the gastrointestinal tract in dogs are connected to dietary fluctuations, and these changes may be recognized by amplifying the 16s rDNA gene and investigating the kinds of bacteria present in the GIT.

gDNA was extracted and amplified using PCR from each canine fecal sample. The amplified samples were then sequenced on the Anschutz Medical Campus using the Illumina technology before being sent to the lab for examination. The F19_67 canine sample and about 450 additional canine samples were investigated to look for the existence of gut microbial populations in the GIT.

A relationship between variations in gastrointestinal tract microbial communities and changes in water sources was established through this analysis, demonstrating that canines exposed to rural water communities had a more diverse gut microbial community. In contrast, canines exposed to regulated urban water sources had a less diverse community due to urban water processing to remove the presence of bacteria in drinking water.

> **PEER REVIEW AUTHOR**
> The Abstract provides a clear and concise overview of the study's objectives and rationale.

Introduction

> **PEER REVIEW AUTHOR**
> Consider expanding the background section to provide a more comprehensive overview of previous research on gut microbial diversity in canines and its relationship with drinking water sources.

Millions of bacteria exist in the gastrointestinal tracts (GIT) of all animals on the planet. The gastrointestinal system is thought to contain approximately 1010 distinct microorganisms, making it one of the most diverse settings for microbial communities in the body (Gootenberg and Turnbaugh 2011). They are among the world's tiniest life forms, contributing to psychological, nutritional, antiviral, and a variety of other aspects of human and animal anatomy (Kil and Swanson 2011).

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Microbes in the GIT operate as an antiviral barrier, protecting the tract from viruses and assisting in digestion and nutrition extraction, among hundreds of other vital tasks. 2011 (Suchodolski). Changes in the GIT microbial community can be connected to factors such as nutrition, geographical location, and environmental exposure, and these factors can help discover linkages between animals and their GIT microbial populations, as well as how they impact animal behavior and health.

Differences in animal and human nutrition can induce significant changes in the richness of gut microbial communities (Scott et al. 2013), and water source differential plays a significant influence. Due to the lack of water filtering processes commonly utilized in urban drinking water sources, animals that habitually consume more rural water sources are likely to have a more diversified microbial population in the GIT.

This also means that animals who drink from city water sources regularly are likely to have a less diversified microbial population in the GIT due to the presence of chemicals like chlorine and fluorine, which are often added to public drinking water. By collecting gDNA from dogs exposed to various water sources, this study investigates the effects of different types of drinking water on the GIT microbial population and amplifies the 16s rDNA gene to identify the species of bacteria present.

Methods

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Describe the sample size and selection criteria for the study participants in more detail.

Extraction of gDNA from Canine Fecal Matter

Qiagen's DNeasy PowerSoil Kit was used to remove inhibitors and separate genomic DNA from canine fecal samples in preparation for a Polymerase Chain Reaction (PCR) to amplify the 16S rDNA Gene. The extraction and isolation were carried out using the DNeasy PowerSoil Kit method. Cells were lysed using six different types of C solutions, which allowed the gDNA to cling to a synthetic membrane. After that, the gDNA on the membrane was eluted and utilized in the PCR experiment.

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Canine Fecal gDNA Agarose Gel

To see if gDNA was extracted, Agarose Gel Electrophoresis was performed. The Lonza FlashGel Electrophoresis apparatus was utilized, followed by the procedure to observe the isolated gDNA. A small amount of the gDNA solution was deposited in an Eppendorf tube, along with water and a sample buffer, allowing the DNA to be viewed under UV light. The solution was mixed and deposited in the appropriate well of the gel equipment. A current was sent via a gel containing conductive fluid. This enabled the gDNA to move through the charged gel toward the positive end, separating it.

Amplification of the 16S rDNA gene by PCR

A Polymerase Chain Reaction (PCR) was employed to amplify the 16s rDNA gene to detect the presence of bacteria in the canine fecal gDNA sample. During the denaturation step, the double-stranded helixes of DNA were separated using a water bath at 96C. During the annealing step at 54°C, the pre-selected primers were coupled to the target DNA in the upstream direction. Taq-polymerase filled the remaining gaps with nucleotides during the Replication phase at 72°C, resulting in a replicated double strand of DNA. The PCR technique amplified certain variable sections of the 16s rDNA gene, allowing them to be seen in an agarose gel.

PCR Agarose Gel with Canine Fecal gDNA

The 16s rDNA gene was recovered in the canine fecal sample using Agarose Gel Electrophoresis. The Lonza FlashGel Electrophoresis apparatus was employed, and the protocol was followed to visualize the isolated gene. A small amount of the PCR product solution was deposited in an Eppendorf tube with water and a sample buffer, allowing the DNA to be observed under UV light. The solution was mixed and deposited in the appropriate well of the gel equipment. A current was sent via a gel containing conductive fluid. This enabled the gDNA to move through the charged gel toward the positive end, separating it.

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Sequencing of Canine Fecal Matter gDNA Sample

The fecal matter sample was sequenced at the Anschutz Medical Campus and returned to the lab for additional examination. Clonal amplification and sequencing by synthesis were utilized in the Illumina Next-Generation Sequencing, which meant that the DNA sequences were recognized and put into a nucleic acid chain. Each DNA molecule has its distinct fluorescent signal, allowing it to be recognized in the nucleic acid chain. These signals aided in establishing the DNA sequence's kind and order.

PEER REVIEW AUTHOR
Discuss how potential confounding factors were controlled or accounted for in the study design, such as diet, age, breed, and geographical location

Analysis of Sequenced Canine Fecal Matter gDNA Sample

Sequenced DNA was returned to the lab and analyzed to determine the correlation between canine gut microflora and canine health.

Results

The appearance of banding on the gel in the equipment verified the presence of gDNA in the canine fecal matter sample. The bands showed the presence of gDNA in the sample, and the gel was used to record the number of bands present and their associated base-pair length.

PEER REVIEW AUTHOR
Present the results in a clear and organized manner, using appropriate tables, figures, and statistical analyses.

The appearance of banding on the gel in the equipment indicated a successful PCR. The existence of bands showed that the target gene (16s rDNA) was effectively amplified, and the gel was used to record the number of bands and their matching base-pair length. Because there was gDNA in the sample that PCR could amplify, the PCR reaction was predicted to function.

Sequencing was successful since the sample had the specified number of reads, as shown in the graph. Overall, the experiment was "successful" since were 479 samples present, indicating that a good proportion of samples exceeded the initial sampling depth threshold value of zero. 335 samples passed the first sampling depth criterion of 1000, 258 samples passed the second sampling depth threshold of 3000, and 194 samples passed the third sampling depth barrier of 5000 when filtered by the sampling depth threshold.

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These values show intermediate results since many samples passed the first depth threshold, but a much lower number of samples met the second and third depth threshold criterion.

The PCR sequencing produced 21791 quality reads for the F19_67 sample that were filtered from the overall number of reads recorded by the Illumina Sequencing System. The overall experiment produced 846 different phylogenetic sequences, and a blast of these sequences produced several different types of bacteria commonly found in the GIT.

The Prevotellaceae family, which is commonly found in the human gastrointestinal tract and can be cultured from the rumen and hindgut of sheep and cattle, the Faecalibacterium genus, which is a commensal bacteria that are commonly found in the gut microbiota and is responsible for the fermentation of butyrate, and the Lachnospiraceae family, which is commonly found in the rumen and gut microbiota.

The existence of these bacteria demonstrates that they originated from a gastrointestinal tract sample; hence, it is assumed that the sequences generated are congruent with gut bacteria. Sample replications were included in the research for the F19_67 fecal sample. The most abundant species of the unknown genus and species was found in the Fusobacteriaceae family, with 5256 reads, making up 24.1% of the sample. The second most abundant species was found in the Bacteroides genus of unknown species, with 1726 reads making up 7.9% of the sample. The third most abundant species was the Clostridium hiranonis, with 1234 reads, making up 5.7% of the sample.

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Ensure that the data analysis methods are adequately described, including the statistical tests used and any adjustments made for multiple comparisons.

When the samples were compared to the kind of water source, there was no discernible pattern in the data, implying no association between the variable and the findings. The Bray Curtis PCoA plot (graphic 3.0) compared sample dissimilarity, and the lack of clustered data points in the graphic indicates that no sample patterns were seen when sorted by water source. The Shannon Index Box Plot contrasted bacterial richness and representation, as well as the overlap of lower and higher quartiles, and almost the entire mean line reveals that no trends were identified when the samples were sorted by water source.

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Discuss the significance and implications of the observed differences in gut microbial diversity among different drinking water sources.

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Discussion

The lack of trends in the data indicates no relationship between water type and the variety and richness of gut microbial populations. The randomly distributed data points in the Bray Curtis Dissimilarity graph (Figure 3.0) and the unaltered median values on the Shannon Index Graph (Figure 4.0) illustrate the data's absence of patterns.

PEER REVIEW AUTHOR
Analyze the findings in the context of existing literature on gut microbial diversity in canines.

This might be due to the study's tiny sample size of roughly 450 dogs, which gives a small data pool to assess trending trends. Error sources might include a lack of gDNA in the sample or a lack of PCR product, which would prohibit samples from being synthesized and impede trend analysis following synthesis. A larger sample pool will be available in the future.

PEER REVIEW AUTHOR
Address any limitations or potential sources of bias in the study design, such as the lack of a control group or the reliance on owner-reported information

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PEER REVIEW AUTHOR
Provide a concise summary of the study's main findings and their potential implications

References

Gootenberg D, Turnbaugh P. 2011. Humanized animal models of the microbiome. Paper presented at: COMPANION ANIMALS SYMPOSIUM; College Station, TX.

Kil D, Swanson K. 2011. Role of microbes in canine and feline health. Paper presented at: COMPANION ANIMALS SYMPOSIUM; Champaign, IL.

Scott K, Gratz S, Sheridan P, Flint H, Duncan S. 2013. The influence of diet on the gut microbial community. Pharm Research [Internet]. [cited 19 Nov 2019]; 69(1):52-60. Available from:

Suchodolski J. 2011. Microbial and gastrointestinal health of dogs and cats. Paper presented at: COMPANION ANIMALS SYMPOSIUM; College Station, TX.

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