Efficacy of oral clioquinol as a depletion method of the gut microbiome of mice

Veterinary Research Scholars Program
University of Missouri

Kristen Rekus[1], Macy Ryan[2], Rhys Dunn[3], Aaron Ericsson[4]

University of Missouri CVM[1], University of Missouri[2,3], University of Missouri Metagenomics Center[4]

Metagenomics Center
University of Missouri

Introduction

- In microbiome research, germ-free and pseudo-germ-free mice are used for fecal microbiome transfers.
- Pseudo-germ-free mice have their gut microbiomes (GM) depleted through the addition of antibiotics to their water.
- Limitations of this practice include water avoidance, due to palatability issues, or incomplete depletion results.
- Therefore, there is a need for an alternative that is tasteless, odorless, and more effective than what currently is available.
- Clioquinol is an oral amebicide that is indicated for idiopathic diarrheal syndromes in horses.
- In healthy, adult horses, a significant decrease in the microbial richness of their hindgut was observed.

Methods

- CD-1 mice housed in groups of 3 with the same sex, treatment group, and GM type (**Figure 1**)
- 24 g of standard chow with 4 mg of Clioquinol for 4 consecutive days was fed = 1 mg of Clioquinol per day (**Photo 1**)
- DNA was extracted from the fecal samples using QIAamp PowerFecal Pro DNA kit
- DNA content determined by fluorometric assay by Qubit
- Libraries of 16S rRNA V4 region amplicons generated and sequenced on an Illumina MiSeq
- QIIME2 was used to filter, assembly, and annotate DNA sequences

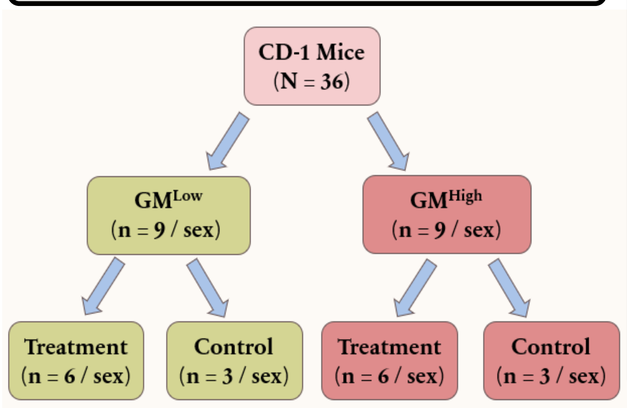

Figure 1. Graphic showing the distribution of mice used in this study.

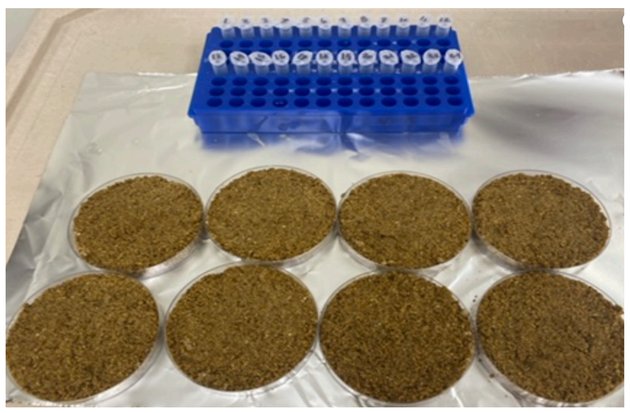

Photo 1. Image showing the collection tubes used for samples (top blue rack) and the Clioquinol mixed with the chow for the treatment mice.

Results

No difference in body weight during treatment

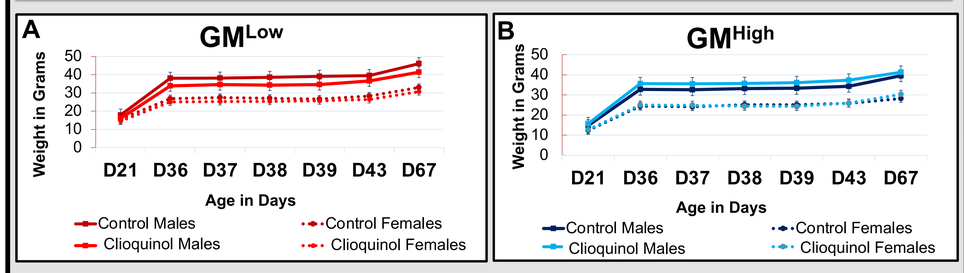

Figure 4. Clioquinol had no effect on weight independent of microbiome type. Line plots showing mean (+/- SE) body weight in CD-1 mice colonized with low-richness GM (GM^{Low} , **A**) or high-richness GM (GM^{High}, **B**) at day 21 (**D21**) of age and subsequent indicated days.

No difference in microbial richness

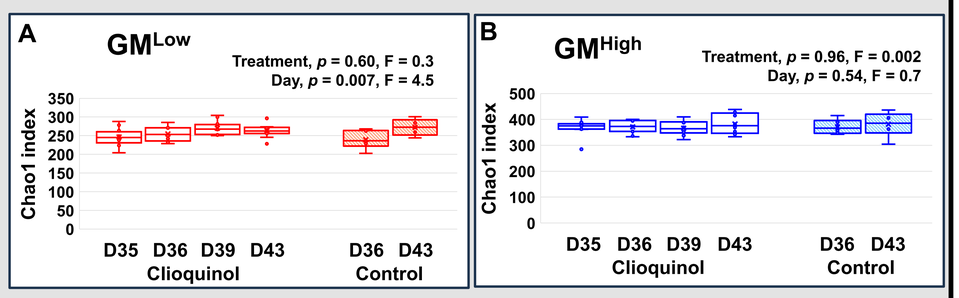

Figure 5. Clioquinol had no effect on microbial richness independent of microbiome type. Box plots showing Chao1 index in Clioquinol-treated and control GM^{Low} (**A**) and GM^{High} (**B**) mice at D35-D43.

Gut microbiome taxa

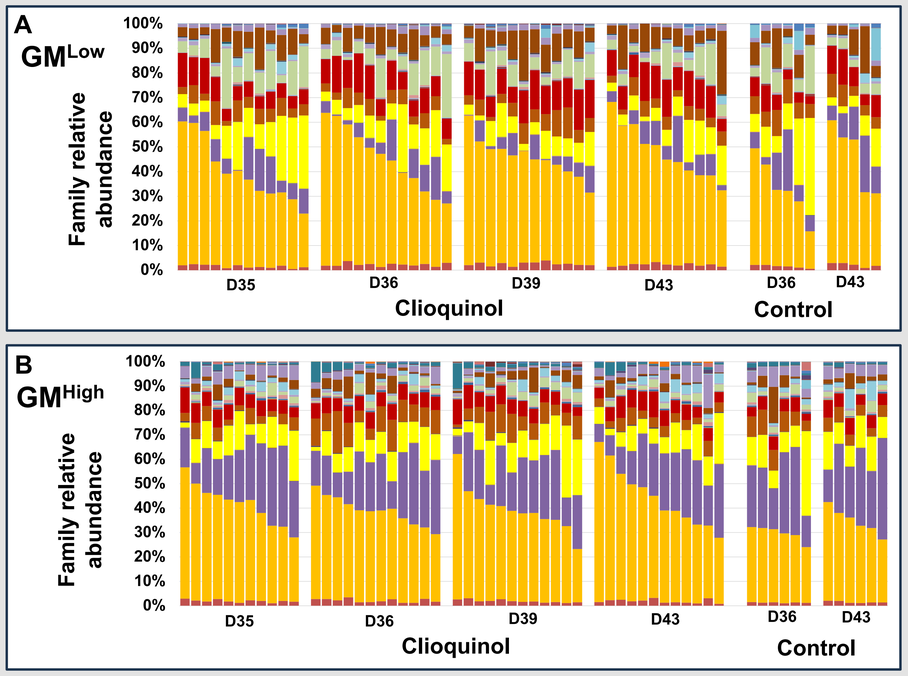

Figure 6. No significant changes in the gut microbiome taxa was seen in either GM^{Low} (A) or GM^{High} (B). Stacked bar plots of the resident bacteria at the taxonomic level of family in each microbiome type and experimental group over the course of the study.

No difference in microbial beta-diversity

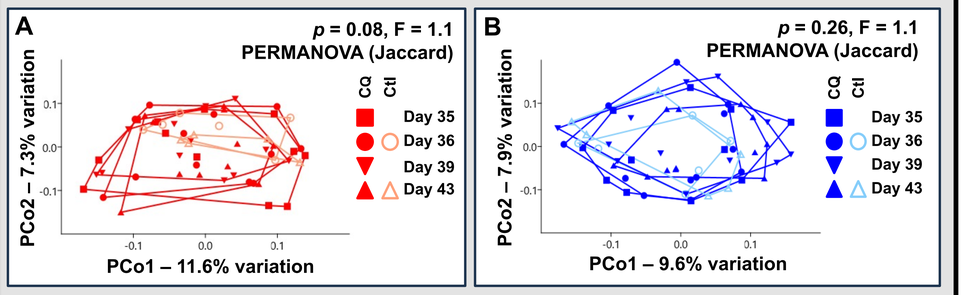

Figure 7. No difference in beta-diversity independent of microbiome type. PCoA plots showing the beta-diversity in Clioquinol-treated and control GM^{Low} (**A**) and GM^{High} (**B**) mice at D35-D43.

Potential selective effects

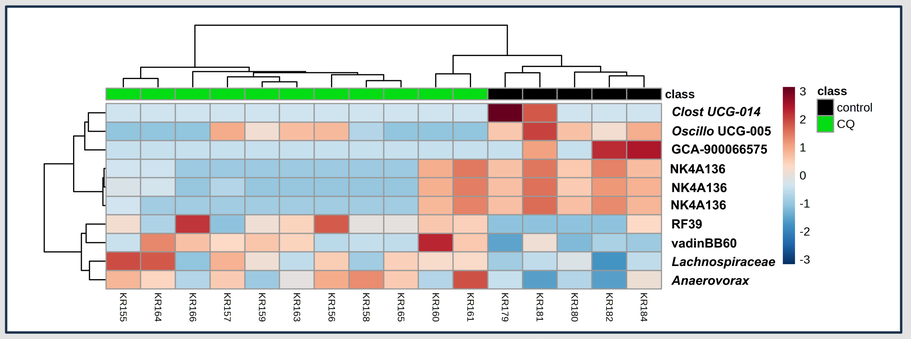

Figure 8. Heatmap showing samples collected from GM^{Low} mice on day 43 and arranged based on hierarchical clustering of abundance of 10 amplicon sequence variants with lowest *p* values (t test).

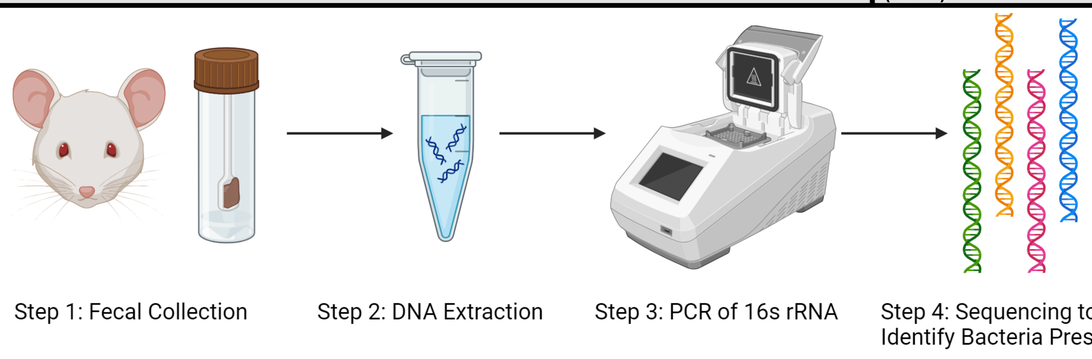

Figure 2. Graphic showing the methods of sampling and processing of the data to identify the number/taxa of bacteria present during treatment of Clioquinol. Made with BioRender.

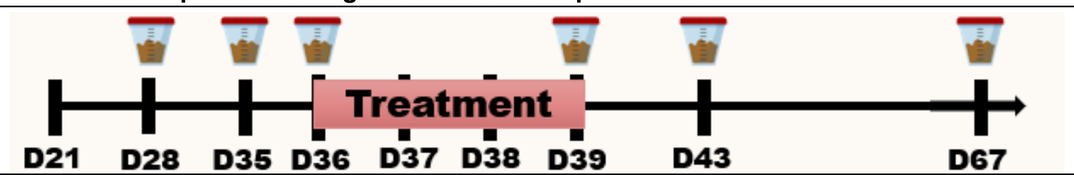

Figure 3. Timeline of the study with age in days of the mice starting from day 21 (D21, weaning) until the last sampling at day 67. Fecal samplings were collected at each indicated day with the fecal cup icon.

Conclusion

The data suggests that treatment of mice with Clioquinol had **no effect** on the GM independent of GM type.

Areas of continuing research to understand why this did not work:

- **Form of drug delivery**
- **Concentration-dependent or time-dependent drug**
- **Physiologic interference**

Acknowledgements

Thank you to Boehringer Ingelheim for the student support of this project; Zachary McAdams; UM Genomics Technology Core; Rebecca Dorfmeyer; and the MU Mutant Mouse Resource and Research Center for providing the mice.