Leptospira, *Giardia spp.*, and *Cryptosporidium spp*. across a Land Use Gradient

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INTRODUCTION

Understanding factors that drive the distribution of waterborne pathogens can help mitigate infection risk. This project aimed to determine the distribution of three waterborne pathogens, *Leptospira*, *Giardia*, and *Cryptosporidium* along a land use gradient in two watersheds in Oregon. Understanding how these waterborne pathogens are transmitted is key to understanding distribution. *Giardia* and *Cryptosporidium* are both transmitted through feces while *Leptospira* is transmitted through urine. *Escherichia coli* is a gram-negative bacillus that is part of the normal GI flora in the lower intestinal tract and can be used as a measurement of fecal contamination in water. This research is an ongoing One Health Initiative Project with data available from last year to review and analyze for any trends.

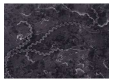
Figure 1. High-resolution scanning electron micrograph of *Leptospira interrogans*


Figure 2. *Giardia duodenalis* trophozoites in Giemsa stain

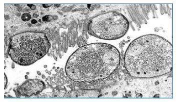
Figure 3. Cryptosporidium parvum electron microscope

OBJECTIVES

- Understand the relationship between waterborne pathogen distribution and the land use patterns
- Determine if the presence of waterborne pathogens is correlated with water quality as measured by Total Dissolved Solids (TDS) and *E.coli* colony counts
- Determine if the abundance of *Escherichia coli* in water samples will positively correlate with the presence of waterborne pathogens.

STUDY DESIGN

- Water was sampled from 24 sites along Marys River in the Willamette Valley, Oregon and 15 sites along White River in north-central Oregon in low, medium, and high disturbance areas.
- Sites were designated as low, medium, and high disturbance based on surrounding land use.
- Temperature, total dissolved solids (TDS), and pH was measured at each site with a water meter, and *E. coli* was isolated and enumerated at each site as a measure of water quality.
- 1 L of water was collected at each site for pathogen detection and filtered with a 2 µm Nalgene filter.
- Filters were divided and part was processed so that the filtered debris was preserved in a formalin buffer for an immunofluorescence assay.
- DNA was extracted from the other portion of the filter for digital droplet PCR (dPCR) detection.
- Merifluor® sample kits were used to test for the presence or absence of *Giardia* and *Cryptosporidium* in the formalin preserved filter debris using direct immunofluorescence for detection via FITC-labeled monoclonal antibodies that attach to the pathogen's cell wall.
- Slides were examined with fluorescent microscopy for the characteristic shape and size of the water pathogens as well as the green color with positive and negative controls used as reference.
- dPCR was used to detect the presence of *Leptospira* in the samples. dPCR protocols for *Giardia* and *Cryptosporidium* are under development

Figure 4. *Giardia* size is typically 10-15 µm and *Cryptosporidium* size is typically 5 µm

RESULTS

Distribution of *Giardia, Cryptosporidium,* and *Leptospira* in the Marys and White Rivers

- Merifluor® assays indicated that three sites were positive for *Cryptosporidium* and/or *Giardia* at Marys River Watershed (Figure 5).
  - *Giardia* and *Cryptosporidium* were detected at Marys River North Fork 1, a low disturbance site.
  - *Giardia* was detected at Sketchy Bridge, a medium disturbance site as well as Herb's Garden, a high disturbance site.
- White River Watershed sample sites did not have any positive results for *Cryptosporidium* and/or *Giardia*.
- No sites were positive for *Leptospira* via dPCR.

A. B.

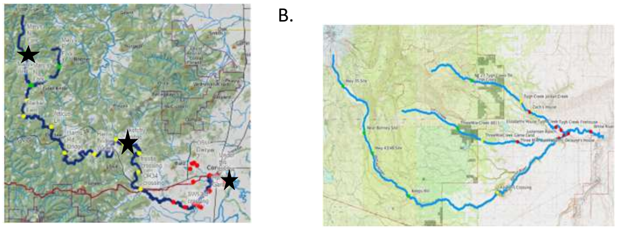
Figure 5. Maps of collection sites along the A. Marys River and B. White River watersheds. Color of dots indicates level of disturbance: green = low, yellow = medium, red = high. Black stars indicate the presence of *Giardia* and/or *Cryptosporidium*.

Water Quality in the Marys and White Rivers Across a Land Use Gradient

- There was no significant difference in TDS among disturbance sites ($F_{2,35}$=1.985, p=0.1525), watersheds ($F_{1,35}$=0.685, p=0.413), and the interaction between disturbance sites and watersheds ($F_{2,35}$=1.407,p=0.258) (2-way ANOVA) (Figure 6).
- There was no significant difference in *E. coli* counts among disturbance sites ($F_{2,34}$=1.012, p=0.374), water sheds ($F_{1,34}$=1.91, p=0.176), and the interaction between disturbance sites and watersheds ($F_{2,34}$=2.473, p=0.099) (2-way ANOVA)(Figure 7).

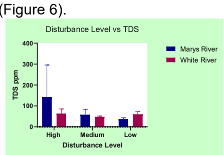

Figure 6. TDS measurements at both watersheds in high, medium, and low disturbance levels. Error bars indicate standard deviation.

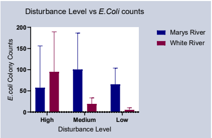

Figure 7. Number of *E. coli* colonies grown in samples collected from two watersheds according to the level of disturbance at each site. Error bars indicate standard deviation.

Relationship between Water Quality and Protozoal Pathogens

A.

B.

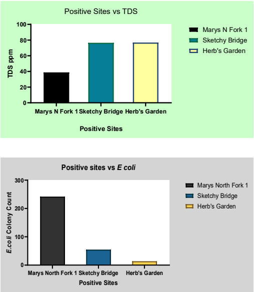

Figure 8. Number of A).TDS and B). *E. coli* colonies measured at three sites that also tested positive for protozoal pathogens.

- Since few sites tested positive for protozoal pathogens, statistical analysis is challenging, but values at each site are shown in Figure 8.
- There was no difference between either TDS (T-test: p=0.7979) or *E. coli* counts (T-test: p=0.8146) when compared to sites positive or negative for protozoal pathogens (Figure 9).

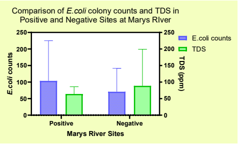

Figure 9. Comparison of colony counts (blue) and TDS at sites either positive or negative for protozoal pathogens. Error bars indicate standard deviation.

DISCUSSION

- Waterborne pathogens are present in Marys River with three sites testing positive.
- When PCR protocols for *Giardia* and *Cryptosporidium* are developed, we will have an additional confirmatory test so it is likely that we could find more positive sites. PCR will also enable us to determine what lineages or species of pathogens are present in the watersheds and thus allow the inference of hosts present and if humans are at risk.
- It was hypothesized that waterborne pathogens transmitted through fecal contamination would be positively correlated with *E. coli* counts, as a measure of fecal contamination. However, we did not find this pattern in our data, possibly due to the low number of positive sample sites.
- Marys River has higher levels of fecal contamination in the medium and low disturbance sites as compared to White River. Both rivers are similar at high disturbance sites.
- Previous year's data shows higher colony counts in 2022 than in 2023 for both watersheds but the overall pattern was similar.
- Water collection in both 2022 and 2023 was limited to 1 L per sample site. In future collections, backpack filters will be used on-site to increase sample size.
- There were only four medium and four low disturbance sites sampled at White River as compared to eleven medium and four low disturbance sites sampled at Marys River. In future collections, an equal number of sites sampled from each disturbance level will help increase the study analysis power.

CONCLUSION

- No correlation was found between land use gradient and water pathogens *Giardia* and *Cryptosporidium* using Merifluor® immunofluorescent assay for detection.
- *Leptospira* was not detected along any site using dPCR so *Leptospira* was not used in any further data analysis.
- There is a positive relationship but no statistically significant correlation between *E. coli* colony counts and *Giardia* and *Cryptosporidium* in the positive sites.
- TDS values were overall greater in the negative sites as compared to the positive sites with no statistically significant correlation found.

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REFERENCES

- Centers for Disease Control and Prevention. (2019). *CDC - DPDx - Giardiasis*. CDC. https://www.cdc.gov/dpdx/giardiasis/index.html
- Humagain, S. (2021, May 4). *Cryptosporidium parvum (Morphology, Life cycle, Pathogenesis, Clinical manifestation, Lab diagnosis, Treatment and Prevention and Control)*. Online Science Notes. https://onlinesciencenotes.com/cryptosporidium-parvum-morphology-life-cycle-pathogenesis-clinical-manifestation-lab-diagnosis-treatment-and-prevention-and-control/
- Mohammed, Dr HARAJI & Cohen, Nozha & Hakim, Karib & Aziz, Fassouane & Belahsen, Rekia. (2011). LEPTOSPIRA: Morphology, Classification and Pathogenesis. J Bacteriology and Parasitology ISSN:2155-9597. 2. 2:6. 10.4172/2155-9597.1000120.
- *Parasitology Review 2017*. (n.d.). Www.slideshare.net. Retrieved October 22, 2023, from https://www.slideshare.net/MicrobeswithMorgan/parasitology-review-2017